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THE SPECIAL PHYSICS OF SEGMENTATION AS SHOWN BY THE SYNTHESIS, FROM THE STANDPOINT OF UNIVERSALLY VALID DYNAMIC PRINCIPLES, OF ALL THE ARTIFICIAL PARTHENOGENETIC METHODS.

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The genesis of this paper is a twofold one. In the first place the careful perusal of the literature which has appeared in very recent years on the matter of the obtaining of artificial parthenogenesis in various forms by a number of methods, and which includes also varying theories of the process of segmentation as interpretations of these data, this perusal readily convinces one that such a consistent and far-reaching synthetic view of the nature of segmentation as known data would seem to warrant one in trying to obtain is quite lacking. In fact no attempt seems to have been made to show that all the methods employed must result in bringing about one and the same series of physical events in the cell preceding and during segmentation, to which the process resulting from normal fertilization is no exception. That vital (?) phenomena can be reduced to a purely physical basis will doubtless be disputed as long as any details connected therewith remain unstudied or in any way ambiguous. The absence of such a complete reduction is in itself, however, no disproof of the correctness of the view as a theoretical standpoint, and success in it will at least always remain a scientific ideal.[1] A clear and detailed demonstration that the effectiveness of the various artificial parthenogenetic methods can be explained if it is held that one series of physical events always occurs in the process of segmentation would seem therefore to go a considerable way in

[1] The position that this standpoint is logically necessary for biology as a science is discussed in the author's article, "The Contrary and the Contradictory in Biology; a Study of Vitalism," in *The Monist*, July, 1903.

the attainment of that ideal. To attempt to do this states accordingly the purpose of this paper.

The second genetic element was the desire to get such a unitary view as at least a preliminary to and if possible a justification of the attempt to initiate segmentation by new methods, viz., by the application of the electrical current to the unfertilized eggs of the starfish, and although these experiments were unsuccessful, the theory, although based on the experimental work of others, is offered for what it may be worth as an endeavor to gain an end, the value of which in itself will not be denied.

1. Experimental Data.

A brief recapitulation of the results already obtained by artificial parthenogenetic methods may, as a preliminary to subsequent discussion, be pardoned.

In the starfish egg parthenogenesis may be produced by: (1) the use of HCl;[1] (2) increasing the osmotic pressure of the surrounding medium;[2] (3) by lowering the temperature;[3] (4) by mechanical agitation.[4] By the first method it is held that the "parthenogenesis of *Asterias* eggs is to be produced by means of specific (hydrogen) ions," at least this is the interpretation of the fact that 100 c.c. of sea water plus 3–5 c.c. $N/10$ HCl acting for from 3 to 20 minutes on the eggs, which are then removed, brings about the desired result.[5] In the case of the second method,[6] although the results are stated somewhat ambiguously, the maximum number of parthenogenetic eggs seems to have been secured by using 15 c.c. of $2\frac{1}{2}$ N KCl + 85 c.c. of sea water at about 23° C. for 15 minutes, then transferring. As for the third, "eggs of *Asterias* may be made to develop parthenogenetically by exposing them for a definite length of time to a temperature of 1°–7° C., in sea water, and then raising the temperature." As an interpretation of this, we find it stated that "the produc-

[1] Loeb, Fischer u. Neilson, *Archiv für die geschichtliche Physiologie*, Bd. 87, 1901.

[2] Greeley, A. W., Biological Bulletin, IV., 3, Feb., 1903, says that Neilson found this method successful.

[3] Greeley, A. W., *Am. Jour. of Physiology*, VI., 1902, p. 296.

[4] Mathews, A. P., *Am. Jour. of Physiology*, VI., II.

[5] Loeb, Fischer u. Neilson, *loc. cit.*

[6] Greeley, *loc. cit.*

tion of artificial development by lowering the temperature is brought about by an extraction of water from the protoplasm, just as if the eggs had been placed in a solution of higher osmotic pressure than that of the sea water," [1] though no explanation of the reason for this is offered. A suggestion as to this is however made by Mathews in his comments on the fourth method, that "the getting of parthenogenesis by agitation may be due to a dissolution of the nuclear membrane, since the centrosome originates close to the nucleus, or it may cause the eggs to lose water like the cells of sensitive plants. The loss of water could be caused only by lowering the osmotic pressure in the cell, and this by decreasing the number of molecules in the cell; and this in turn by synthetic processes." [2]

In other forms artificial parthenogenesis may be obtained by similar or slightly different methods; *e. g.*, in *Arbacia* by osmotic pressure, 50 c.c. $\frac{20}{8}$ *N* $MgCl_2$ or NaCl + 50 c.c. sea water,[3] and at least a segmentation by lack of oxygen, by heat, or by exposure to alcohol, chloroform, or ether;[4] in *Chætopterus* likewise by the use of KCl, KNO_3, K_2SO_4 (2½ *N* + 100 c.c. sea water), NaCl, $MgCl_2$, $CaCl_2$ and sugar,[5] in *Amphitritus* by $Ca(NO_3)^6$ (2 c.c. *N* + 99 c.c. sea water);[6] in *Nereis* by osmotic pressure, (20 c.c. 2½ *N* KCl + 80 c.c. sea water, 30 minutes),[6] in *Podarke obscura* by use of the same solution.[7] As theories and interpretations of the results obtained by these factual methods, we find in addition to those already cited the following, which are quoted in abstract:

"All that the spermatozoön needs to carry into the egg for the process of fertilization are ions, Mg, K, HO or others, to supplement the lack of the one or counteract the effects of the other class of ions in the sea water, or both. The ions and not the nucleins in the spermatozoön are essential to the process of

[1] Greeley, *loc. cit.*

[2] Mathews, *loc. cit.*

[3] Loeb, J., *Am. Jour. of Physiology*, Vol. III., Nos. III. and IX. and Vol. IV., IV.

[4] Mathews, A. P., *Am. Jour. of Physiology*, IV., VII.

[5] Loeb, J., *Am. Jour. of Physiology*, IV., IX.

[6] Fischer, M., *Am. Jour. of Physiology*, VII., III.

[7] Treadwell, BIOLOGICAL BULLETIN, III., 5.

fertilization ;[1] or the spermatozoön may carry enzymes." "Either of these two causes affects the most important qualities of life phenomena, *i. e.*, causes the proteids (1) to change their state, or (2) to take up or lose water."[2] Further details as to these two possible events are not given, however, but it is quite evident that the two may be coincident, so that the latter change may take place in any case.

To summarize systematically, a cell division can be caused in various forms by one or more of the following classes of stimuli: (*a*) mechanical; (*b*) heat (or cold); (*c*) osmotic; (*d*) chemical, or, if one will, ionic; the third for the reason that either of the electrolytes MgCl, or NaCl, or the non-electrolyte sugar, may be used for *Arbacia*;[3] therefore no specific chemical effect is to be accepted here. The fourth, a distinctly chemical effect, is evident, for HCl is effective for *Asterias* eggs and KCl is not; so also only the Ca ion for *Amphitritus*. Here then it is the kation that is considered to cause the segmentation, but that a fundamental chemical effect different to an ionic, *i. e.*, electrical charge effect is present is shown by the fact that, keeping the osmotic pressure and the number of charges on the kation the same, but changing the ions, the effect is different. This is confirmed by the comparison of the action of KCl and NaCl on muscle.[4] A specific chemical effect is therefore not done away with even if the difference in effect is reduced to a difference in the path of the charge moving around the atom. For the cause of this latter difference must in turn be a fundamental difference in the atoms themselves. The same kind of proof of an irreducible and ultimate chemical difference is found in the results of Lillie's work on the effect of Na, K, Ca and Mg salts on *Arenicola* and *Polygordius*, and of Mathews on the different stimulating effects on the nerve of NaCl, NaBr, NaI and NaFl. This fundamental chemical difference is related to the difference in solution tension, as Mathew's work this past summer has shown.

However, not alone the stimuli, the external agents initiating

[1] Loeb, J., *Am. Jour. of Physiology*, III., III.
[2] Loeb, J., *Am. Jour. of Physiology*, III., IX.
[3] Loeb, *Am. Jour. of Physiology*, IV., IV. and III., IX.
[4] Loeb, *Am. Jour. of Physiology*, III., VIII.

segmentation are to be put into the above classes, but also, with the addition of the class, surface energy, which is of special importance here, the phenomena taking place within the cell itself, preceding and during cleavage. We accordingly consider the cell to be a physico-chemical object, whatever else it may be, and subject therefore to general physical principles. The special nature of the physical processes that occur in it is to be demonstrated by showing that the effectiveness of the physical methods used for causing segmentation implies that, or at least can be explained, if by each method only one and the same series of events is made to take place. The bringing together of results in this way exemplifies what we have termed synthesis, and the internal agreement with which it is identical makes for the probable correctness of our theory.

2. General Physical Principles to be Observed in the Interpretation of These Data.[1]

If the energies both within and without the cell belong to the classes named, we must in our endeavor to get at the meaning of the data at hand be guided strictly by the most general fundamental chemical and physical principles valid for those. These principles, some of which are of course well known, may be stated as follows:

I. The "first law" of energetics, that of the conservation of energy. This is considered to have an experimental basis in the fact that, *e. g.*, a weight of one kilogram falling 424 meters raises the temperature of one kilogram of water 1° C. as indicated on an arbitrarily selected scale. This is interpreted to mean that the kinetic energy of the falling body is quantitatively equal to the heat energy gained in the rise in temperature. However this cannot be strictly proven, for the two energies are qualitatively different, and have no common factor. It would therefore be

[1] The principles as stated are to be found in no one author, but are with their criticism the result of the study of the works of Planck, Mach, Ostwald, Helm, Wald, Riecke, and others; some of these are as follows: Planck, "Prin. d. Erh. d. Energie"; Ostwald, "Vorlesungen über Naturphilosophie," "Allgemeine Chemie," and other writings; Helm, "Die Energetik nach ihrer gesch. Entw."; Rankine, *Philos. Mag.*, 1867 (4); Mach, "History of Mechanics," "Wärmelehre," "Pop. Lect.," "Analyse der Empf"; Riecke, "Lehrbuch d. Physik."

quite as logical, though not as practical to interpret the two as quantitatively different. To interpret as equal is therefore to base the law of conservation on an assumption not proven, yet not disproven.[1]

In the second place the law is based upon the impossibility of a system's continuing to do work unless energy is received from without. In this the assumption is implicitly contained that work cannot be created *ex nihilo*. Were it possible, however, for a system to receive from without the work (energy) which it itself does, a perpetual motion would be possible.

II. The second law prevents this. In every "Ausgleichung" or transformation (Umgleichung) of energy, some heat is produced, only part of which at best is again available, for the reason that it tends to "dissipate"; it cannot pass from the body of the lower to that of the higher potential (temperature) but only conversely. The entropy of the universe is accordingly said to increase. This characteristic of heat energy is a special case of a law (the second) valid for all the energies. Its meaning is that all events have a definite direction.

According to the first law then if one form of energy disappears another form or forms held to be quantitatively equal to it must appear. Energy may therefore be defined as that which in changing conserves itself. Implicit in both the first and second laws is the definition of it as that which does work, but that there are objections to this is evident from the facts stated in the second, that some energy in the form of heat with no difference of potential (T) cannot do work.

III. The factors of energy. Already present though unrecognized in the early development of mechanics, but made explicit first in thermodynamics, and later extended to all forms of energy is the view that each is made up of the product of two factors, a potential or intensity, on the one hand, and an extensity or capacity factor, on the other. In heat energy these factors are respectively temperature and entropy Q/T (specific heat), in kinetic energy V and MV, in volume energy (gases and solutions)

[1] This procedure illustrates the necessary dogmatism of all science, and the superiority of a pragmatic to a logical justification, a subject which will be treated at length in another paper.

pressure and volume, in surface energy surface tension and surface, in chemical energy chemical intensity (avidity) and mass.

IV. The law of events or action. Every event, *i. e.*, the going over of energy from one body to another or the transformation into another form is conditioned (*a*) not by the absolute quantity of energy involved, but by the potential factors which (*b*) must be opposed to each other in direction and be of different value, *i. e.*, a potential difference must exist; (*c*) this potential difference must not be compensated by a third potential, *i. e.*, must be uncompensated. Unless these three conditions are fulfilled a system remains in its state of equilibrium; if they are given between a system and its environment work is done either on it or by it, the amount of which depends upon the product of the total extensity into the potential difference. If such conditions are held to exist wholly *within* a system so that change occurs in it, this is equivalent to dividing the system into environment and smaller system, the limitation of which in every case is arbitrary though for practical purposes necessary.

V. The *direction* of the energy transfer is always from the higher to the lower potential, the one falling as much as the other rises until equilibrium is reached. By this event however a new potential difference between a second and a third energy form may be created, and with the getting of equilibrium by this a series of events is formed. Assuming the potential of a second energy to have increased or to be *continuously* increasing, the result is that in the "Ausgleichung" between this second and a third within the system, the second may be of the same intensity at the end as at the beginning of this event, while the third shows a rise in potential. This kind of event during which one potential is kept constant is called isocyclic. In comparison to the third the second potential presents here a relative fall. Conversely those events in which the extensity factor remains constant and the intensity alone changes are called adiabatic. Both kinds of changes can be brought about by manipulation of a system which is isolated with the exception of the manipulation. In natural events, however, there is always a change of potential as well as of extensity. The process of getting equilibrium is quite consistent with an absolute rise in two potentials

within a system in relation to a fall in a third potential outside of the system; at the same time the extensity factors of the first two may diminish. Under these circumstances work is done on the system. Conversely, two potentials within a system may get equilibrium and, at the same time, both decrease absolutely in relation to a third external, which rises. The system then does work. Both of its extensity factors may however increase in this process, but this does not necessarily mean a gain in energy, for energy equals potential × extensity.

Concrete instances of these possible cases, which have been selected as bearing directly on our special problem, we shall find in the physical events making up the process of segmentation and cleavage.

3. Surface Tension and Osmotic Pressure.

In the instance of the normal progress of the event of segmentation, *i. e.*, with a cleavage of both nucleus and cytoplasm, no matter whether this is the result of natural or artificial fertilization, it is an undeniable fact that, coincident with and as a culmination of all the processes taking place in the cell there is a decrease in surface tension in at least certain parts of the surface. This follows necessarily from the change in the radius of curvature of the approximately spherical form of the egg to the increased radii of certain parts of the surface of the *constricted* form. For surface tension, the potential of surface energy, varies inversely with the radius of curvature, and is greatest in the spherical form. From this it follows that in cleavage there is in any case an *average* decrease in surface tension accompanying the redistribution of this. The very fact that these changes occur may be advanced as a proof that in protoplasm we are dealing with either a solution or a fluid. Accordingly the surface energy is to be considered as due to or identical with the "attracting forces" (cohesion) of the fluid particles. This redistribution of surface tension may be correlated with and is doubtless confirmed by observed protoplasmic streamings.[1]

It would not, however, occur of itself; it must have an ultimate cause, either within or without the system (the egg), and in either

[1] Cf. Bütschli, "Protoplasm."

case is possible only if it is either the immediate *result* or *cause* of a change in the potential of that energy which is opposed to the surface tension, viz., osmotic, *i. e.*, the "repelling" forces of the substance in solution.[1] The change in surface tension is one part of the "Ausgleichung" of the potential difference existing between it and the potential opposing it, viz., osmotic pressure. The necessary coexistence of these two kinds of energy in a solution and the characteristics of each bring it about however that when one potential decreases the other does also, although there may be a relative fall of one and rise of the other, thus making an isocyclic event possible. For just as the surface tension decreases with an increase in surface and conversely, so also is a decrease in osmotic pressure accompanied normally by an increase in volume (and therefore of surface) and conversely.

It is evident then that in segmentation with its change of form and redistribution of surface tension we are always dealing immediately with the interrelations of two energy forms, surface and osmotic, and mediately with any causes which may act on them, whatever these may be. If we succeed in showing that the various physical agents used for producing artificial parthenogenesis can be effective only by in any case causing changes in either one or both of these energies, then we shall have obtained that unification of evidence which is our purpose.

4. Factors Conditioning Variations and Changes in these Energies.

The existence of an uncompensated potential difference within the egg can be accounted for in two ways. It may be either the result of changes already going on in the egg, *e. g.*, the becoming active of preferments[2] and so the formation of new chemical compounds, processes of maturation, or those leading to "natural death,"[3] but this is the same as saying that such a difference is already there and that it leads to others; or the

[1] In reply to the possible objection that colloidal solutions have no osmotic pressure there is experimental evidence that they have this and are diffusible, thus showing that there is no essential difference between these and other solutions. Cf. Höber, "Physikalische Chemie der Zelle u. Gewebe," s. 43, et seq.

[2] Cf. Hofmeister, "Chemische Organization der Zelle."

[3] J. Loeb, Biol. Bull., Nov., 1902, "Maturation, Death, etc., in Asterias."

result of action between the egg and its environment, in which case the egg may either have done work, as, *e. g.*, in a medium of less osmotic pressure, or work may have been done upon it by means of mechanical agitation, heating, abstraction of water, or fertilization by a spermatozoön. Any of these possible events must take place in complete agreement with the general conditions above outlined, and will in every case of cleavage ultimately condition a change in the relation of two kinds of energy, surface and osmotic.

That this is true is shown by consideration in detail of the characteristics of these two energies. Surface energy is due to the mutual attraction of the molecules of a fluid, which here forms one part, viz., the solvent, of protoplasm. The molecules at a certain distance from the surface are each free to adjust their mean position under the influence of the surrounding molecules, the mean position being that in which each is acted upon equally on all sides, with the result that the mutual attraction is not rendered manifest. At the surface, however, if this be free, or relatively free, as is shown by experiment (*i. e.*, if a chemical difference between medium and egg exists) the molecules are virtually acted upon only by those lying internal to them. The result is a system of forces manifesting their action throughout the fluid and at right angles to the surface, *i. e.*, radially and tending to reduce the surface to the least possible area, *i. e.*, the spherical form. The surface in contact with a chemically different medium so that mixture does not take place, acts like or is in fact a membrane or film.

The factors by which variations in the attraction of these particles for each other are determined are: (1) Their chemical nature, and consequently (2) the density of the fluid, (3) the temperature, a rise in which decreases the tension, (4) the presence of electrical charges.

Osmotic (volume) energy is identical with the mutually repelling forces of molecules in a solvent, and follows the law of gases. Its two factors are accordingly volume and pressure, and in a natural event as the former increases the latter decreases. The conditions upon which variations in this depend are accordingly (1) for equal weights of dissolved substance, and equal

volumes, chemical constitution, (2) nature of solvent, (3) electrolytic dissociation, (4) heat. Changes may be brought by chemical interaction, enzymes, heat, mechanical agitation, or amount of solvent present.

In the egg, which is a system of coëxisting energies, it is then *directly* with the attracting and repelling "forces," *indirectly* with anything conditioning them, that our problem is concerned. To be sure the molecules of both solvent and dissolved substance, the solute, must be admitted each respectively both to attract and to repel. In the former, however, the attracting forces predominate as is shown by the slight effect of temperature; the repelling may therefore be ignored. In the latter the converse is the case, for the same reason.

The form and surface of the egg at any time is accordingly determined directly by these two energies, and indirectly by any of the above enumerated conditions modifying them, for any uncompensated potential differences which may rise through the action of either internal or external causes must be equilibrated with a resultant change in surface and perhaps in volume.

The valid objection may here be offered, however, that a uniform or average decrease in surface tension does not account for the change in form accompanying segmentation. This can doubtless be met by showing that, at the same time that a decrease takes place, a rearrangement also results because of the existence in the egg of localized chemical differences, *i. e.*, the egg is organized. That these exist is shown: (1) experimentally by the different staining reactions of, *e. g.*, the nucleic and cytoplasmic cells to acid and basic dyes;[1] and (2) by observed morphological differences and changes. Granted this chemical organization, localized changes in it and so of the osmotic pressure necessarily present in such a solution can be brought about either normally by the entrance of a spermatozoön, whether its action be enzymatic or chemical, or abnormally by ions, temperature, agitation, or abstraction of water. Differences in osmotic pressure thus arising may remain localized either because of membranes within the egg, *i. e.*, of further chemical differences, or by reason of the relatively slow diffusibility of colloidal particles.

[1] Cf. the researches of Picton and Linder.

The chemical changes in the solute to which they are due may in turn be accompanied by electrolytic phenomena, and so by an attraction of unlike and repulsion of like charges, so that with all of these factors taken together reasonable grounds are furnished for the understanding of the presence of irregularities in the cleavage form. The fact of this constricted form and of the possibility of its explanation on the above basis must be constantly kept in mind in the subsequent considerations. If then the final event in the series of changes leading up to segmentation is that ending with a change of form and average decrease and rearrangement of surface tension, after which a "resting stage" of relative equilibrium exists for at least some time, then this series of events must have resulted from or be in part identical with a disturbance of equilibrium within the cell before the event of cleavage. The various theoretically possible ways in which this disturbance can be brought about must therefore be presented systematically and in detail.

5. Possible Ways of Creating a Difference Between the two Potentials Concerned, Pressure and Tension.

There is, first, the possibility that the surface tension may be greater than the osmotic pressure of the moment can withstand, *i. e.*, that a potential difference in the direction of surface tension — osmotic pressure has been formed. This of course is equalized with the establishment of equilibrium, but therewith both extensity factors, surface and volume must have decreased and the cell have lost water, while both intensities, tension and pressure, however, have presented an absolute increase. A potential difference in this direction might theoretically be caused in two ways, either (1) by increasing the surface tension or (2) by decreasing the osmotic pressure, in each case keeping the other potential constant. Conversely, if the osmotic pressure is first increased, *e. g.*, by an analytic chemical process resulting from oxidation, or if the surface tension is first decreased by, *e. g.*, electrical charges, then a potential difference in the direction of osmotic pressure — surface tension will exist. This being uncompensated, the resulting process of equilibrating is identical with the taking place of an increase in both extensity factors and

a decrease in the intensities; but in the equilibrating of potentials the decrease in the tension is less than in the pressure, for the former is already lower; it therefore presents a relative rise.

This scheme offers a systematic mode of procedure for taking up the examination of the various methods of producing segmentation and development. However, since we are concerned only with the bringing about of an average decrease in surface tension, we shall use only the second class of possibilities.

A. Factors Decreasing Surface Tension Directly.

One theoretically possible way of decreasing the tension directly is by means of either a rise in temperature or by bringing to the surface (the poles of the egg) like electrical charges.

The attracting forces, it has been seen, may be regarded as due exclusively to the solvent, which for protoplasm, we have every reason to believe, is only water; consequently any direct decrease in surface tension by changing the solvent *chemically* seems to be excluded for practical reasons. But a rise in temperature lessens the attraction potential directly at the same time that it increases the osmotic pressure; therewith is equilibrium done away with, work being done on the egg from without. The pressure being too great for the tension, the volume must increase, with absorption of water, until equilibrium is established. The extent of surface therewith increases, but the potential, surface tension, decreases absolutely, although, relative to the pressure opposing it, it rises and the egg thus in turn does mechanical work by displacing the surrounding medium. The *direction* of these events is thus determined by the difference in potential.

With this theoretically possible course of events agrees exactly the observed swelling and liquefaction of cells when heated,[1] as well as the starting of development in *Arbacia* by a rise in temperature, observed by Mathews.[2] This constitutes the first example of factual methods agreeing with our synthetic point of view, and indicates that liquefaction consists either in increasing the pressure by analytic processes, or in absorption of water, or in both.

[1] Greeley, A. W., BIOLOGICAL BULLETIN, IV., 3, 1903; V., 1, 1903.
[2] *Amer. Jour. Physiology*, IV., VII.

Such liquefaction phenomena do not of course in every case mean, nor are they the whole of cell division, neither do they in any case account for this except by also taking into consideration the chemical organization previously referred to. But that such a liquefaction takes place during segmentation is evidenced also by (1) the greater susceptibility of the fertilized egg at that time to ether, HCl, KCl, etc., results obtained by the author in work on *Arbacia*, and an account of which will appear in a subsequent paper, and (2) by the results obtained by Lyon this summer, which indicated a rhythm in the use of oxygen and the giving off of CO_2 by the egg. The last is at the maximum during segmentation, which accordingly points to a greater splitting of molecules at that time and a consequent increase in osmotic pressure and absorption of water.

Another theoretically possible direct cause of a decreased tension would be the presence at the surface of the egg of like electrical charges. But as this concerns our own experimental work we postpone its consideration until further on.

A *fourth* possible method here would be that of mechanical agitation, by means of which the attracting forces of the membrane of the cell would be lessened; for in a physical experiment, at least, the form is charged, the average tension therefore decreased by mechanical work done from without. By analogy this may hold good of the egg, and to it corresponds the method of agitation used on starfish eggs. Its efficacy may be further explained if it is recalled that agitation may also result in a molecular splitting and consequent increase in pressure. As this completes the possible cases under this class of methods we may pass to the consideration of the other class.

B. Factors Directly Affecting the Osmotic Pressure.

We are here concerned with the establishment of a potential difference in the direction of osmotic pressure — surface tension by directly *raising* the former. Two classes of theoretically possible methods for doing this are to be considered, by bringing about (1) analytic chemical changes in the solute and (2) physical changes of pressure.

I. *Chemical Changes in Colloidal Particles by (a) Chemical Methods.*

Other conditions such as the amount of solvent being assumed as constant, the first class of changes can in theory be caused by any agent which increases the number of molecules or of particles, *i. e.*, which causes a splitting up of these within the egg. For, as is well known, in a given volume the pressure varies directly with the number of molecules. Under these circumstances, in order that subsequently there may be equilibrium between pressure and tension, both potentials must decrease, the latter however presenting a relative rise; at the same time both the volume and surface increase, water is absorbed, and the medium outside the cell is really of smaller volume and greater pressure than before.

This series of events agrees with that which we have seen must take place in segmentation, viz., an average decrease of tension. Confirmatory of the correctness of our theory and quite agreeing with it are a number of experimental methods for obtaining artificial parthenogenesis when the direct effect can be only chemical or ionic or both, but in any case not purely electronic. Thus in *Asterias* eggs HCl is effective, but not KCl, from which a specific H ion effect is inferred.[1] So also are Ca ions alone effective for *Amphitritus*.[2] (The effect of the hydroxyl ion, or of O, can have only the same direct chemical effect primarily; by these conditions segmentation is retarded or exhibited, thus indicating that for segmentation analytic processes are essential; for, *e. g.*, oxygen in the presence of ferments means a splitting up of molecules.) The accepting of a *specific chemical effect* of these ions is not invalidated by the admission of an "electronic" effect also, but is necessitated by just such data as the above, which are the results of an application of the "method of difference." Just how this chemical effect is caused, whether directly by action on the protoplasm, or indirectly by first making certain "preferments" in the egg active cannot be definitely stated, but this deficiency does not in itself alter the correctness of the above view. The effectiveness of another practical method is thus found

[1] Loeb, Fischer and Neilson, *Archiv für geschichtliche Physiologie*, Bd. 87, 1901; previously cited.

[2] Fischer, *loc. cit.*

to be explainable upon the basis of our theory and the usefulness of the attempt to synthesize therewith demonstrated.

From exactly this same standpoint also can the effect of an entering spermatozoön be explained, whether its immediate action be chemical or enzymatic, and thus the physical processes of normal fertilization and segmentation made clear, at least in part.

(*b*) *Chemical Changes by* (*b*) *Physical Means.*

Theoretically possible physical means for producing this splitting of particles or of molecules are heat, and mechanical agitation; the former, as a rise in temperature, may be supposed to have this direct effect in agreement with well-known phenomena in pure chemistry, or, as a preliminary to this, to first make certain enzymes in the egg active; or it may be considered to have a purely physical effect, for since $PV = RT$ it directly increases the pressure and decreases the tension. All of these three effects may and probably do coexist. Agreeing with these theoretical possibilities is the practical one of starting development in *Arbacia* by the action of heat (rise in T).

In the same way can also the practical method of causing parthenogenesis, *e. g.*, in *Asterias* eggs by mechanical agitation,[1] be explained, *i. e.*, in analogy to facts in chemistry. For example, in the "diazo-compounds" mechanical shock starts analytic processes. Such compounds are accordingly said to be "metastable." At the same time that the pressure is increased by such analytic processes the potential difference thus formed may be further augmented by a decrease in the tension also caused by agitation, as pointed out above.

The effectiveness of this entire class of methods, both physical and chemical, for causing analytic changes in the solute may be explained as follows: Work is first done on the cell in strict accordance with the general physical principles stated. As a result of the analytic chemical processes taking place, the pressure is at first increased, possibly also the tension decreased at the same time. If the potential difference thus created is uncompensated by a third, *i. e.*, if the pressure of the surrounding medium has been kept constant, *e. g.*, by retransferring the eggs to sea

[1] Mathews, *Am. Journal of Physiology*, VI., 2, 1901.

water in the HCl-*Asterias* method, *i. e.*, if another potential difference exists than in the cell, the latter must in turn do work; its two potentials accordingly decrease, the extensity factors increase, water is absorbed, and as a result of new localized chemical and osmotic differences in the egg plus accompanying electrical changes, the constricted form of cleavage appears.

(*Semipermeable Membranes.*)

In the above presentation the assumption is implicitly made that the surface of the cell acts as a semipermeable membrane, so that, first, only specific ions can enter, and second, others, those within the cell cannot make their exit, while water can pass either way. Only on these conditions can osmotic pressure be truly exerted.

The question accordingly arises, if this possibility is done away with if the so-called membrane is due to, or identical with, only the surface tension of a fluid in which colloidal particles are in solution. The answer to this on the basis of the evidence at hand and to accept the position generally taken is negative, but again brings us to the matter of chemical organization. Rhumbler[1] has shown experimentally that particles in the surface are not freely displaceable, nor do protoplasmic streamings to the outside take place; this can be accounted for only by assuming a foam structure and a state of solution. This means that the apparent membrane is a result of the forces of surface tension. Furthermore, with very probable reasons for the surface being chemically different to the interior as it is to that of the surrounding medium, and with the known fact of the difficult diffusibility of colloidal particles, it is quite intelligible that at the same time that a displacement to the interior is rendered difficult the specific chemical nature of the surface film should prevent the passage through it of some ions while allowing that of others, quite analogous to the artificial semipermeable membranes of Pfeffer. The same explanation would hold good for any other apparent membranes, like that of the nucleus, within the cell whose morphological differentiation can be established.[2] All these factors,

[1] Rhumbler, "Aggregatzustand u. Physikalische Besonderheiten des Zellinhaltes," *Zeitschrift für allg. Physiologie*, I., 3, '02.

[2] Cf. Höber, *loc. cit.*, p. 47, for confirmatory evidence.

viz., local chemical differences, membranes within the cell, difficult diffusibility of colloidal particles, splitting of molecules, localized processes, resulting electrical changes, make the constricted form of the egg at cleavage with its rearrangement and average decrease of tension not only intelligible, but *a priori* probable.

II. Means for Changing the Osmotic Pressure Physically.

Purely physical changes in the repelling forces of the cell are possible, and the theoretical methods to be inferred from these are confirmed by a number of practical methods for producing artificial parthenogenesis.

(*a*) *Hypertonic Solutions.*

The first of these both theoretically and also practically possible methods is the direct raising of the pressure within the cell by first surrounding it with a hypertonic solution and then subsequently transferring it to the sea water whereupon segmentation takes place. This method is used for *Arbacia*, *Asterias*, *Chætopterus*, *Nereis* and *Podarke*, and is perhaps the most important of the artificial parthenogenetic means. Its effectiveness may be explained as follows: In a hypertonic solution there is at first an uncompensated potential difference between the pressure within and without the egg, which is equalized by the passing of water through the membrane of the egg to the medium. The relative number of molecules of solute within the egg is accordingly increased. At the same time with a decrease in the egg's radius of curvature accompanyiug the decrease in size the surface tension has been increasing to compensate the increased internal pressure, though this compensation never quite takes place as long as water continues to be withdrawn, but theoretically is always somewhat behind. The egg thus receives energy from without. With the transferral of the egg to sea water, which is of lower pressure than it is, a potential difference in the opposite direction next exists which is uncompensated by the tension owing to the permeability of the membrane to water; accordingly an event, viz., absorption of water, takes place until the pressure both within and without is the same. In this proc-

ess, however, both the pressure and the tension of the egg *decrease*, as our theory demands, while the volume and surface increase until equilibrium is attained between the two energies within and the pressure without. But therewith new potential differences between these and other energies, *e. g.*, chemical, may have been created, by which the series is continued up through the various stages of development.

However, in this method it is not directly evident why, instead of segmentation taking place, simply the former size of the egg is not recovered when it is returned to sea water. To account for the constriction actually occurring it is again necessary to make the assumption which is nevertheless theoretically justifiable in analogy to well-known phenomena in chemistry, that in connection with localized chemical differences some of the colloidal particles are naturally in a "metastable" condition, which is done away with and chemical changes started by the withdrawal of water, and which therefore cannot subsequently be regained.[1] It may also well be in analogy to known instances elsewhere that so-called preferments become active only on the condition of a certain degree of concentration being present and that, becoming active with the concentration here present, they initiate chemical changes which finally cause the constriction of the cleavage form.

(*b*) *Action of Temperature Changes.*

The second of the class of methods we are here considering, viz., the physical, has to do with variations in temperature. Two possibilities therefore exist, a raising and a lowering of the temperature of the medium and so of the egg.

A raising of the temperature directly increases the pressure, but it may also produce, as has been seen, a molecular splitting either directly or through the mediate action of preferments, and, as before stated, decrease surface tension. Any one or all three of the effects taken together are essential with the formation of a potential difference in the required direction; the starting of development in *Arbacia* eggs by heating may be explained in this way.

[1] Cf. Ostwald, *Vorlesungen über Naturphilosphie*, s. 271 et 353, et seq.

Greeley[1] found however that artificial parthenogenesis could be produced in *Asterias* eggs by exposing the eggs to a temperature of 1°–7° C. and then allowing this to rise, keeping them in the same sea water all the time. This can be explained as follows: A lowering of the temperature directly reduces the osmotic pressure of both medium and egg, yet increases the surface tension. A potential difference within the egg in the direction of tension-pressure is thus created, the equalization of which necessitates a loss of water. This view is confirmed by the spherical form assumed and the losing of water by many species as a result of exposure to low temperatures.[2] The loss of water as a result of the increased contracting forces cannot continue indefinitely for the reason that, by virtue of the increased osmotic pressure caused by it, the egg itself would tend to absorb water. Consequently the *three processes* must be considered to occur until there is equilibrium in the entire "system" of *egg-medium;* likewise they would continue to take place, though in opposite directions, when the temperature was subsequently raised; *i. e.*, the pressure and tension would then both simultaneously decrease and the egg absorb water. Consequently this method would agree with our theoretical demand, that the event of cleavage is always identical with a decrease in surface tension.

(*c*) *Electrical Changes.*

The third possible way of bringing about changes in the egg which are in themselves physical (electrical), but perhaps due directly to chemical causes as we have indicated, is by the use of electrolytic methods. These changes may have to do with both pressure and tension at the same time and we have referred to them previously as perhaps to be necessarily considered as present in any case in order to account for the constricted form of cleavage. For it can be shown that every method that we have analyzed may, at the same time that it results in the changes in chemical composition, pressure and tension, have also accompanying these a change in the electrical condition of the egg.

[1] *Am. Journal of Physiology*, VI., 1902, p. 296.

[2] Greeley, *Am. Journal of Physiology*, 1901, VI., p. 122; BIOLOGICAL BULLETIN, III., p. 165 and V., p. 42.

Lillie[1] in two recent papers has emphasized the importance and essentiality of these electrical phenomena for segmentation, but, inasmuch as he ignores to a certain extent the consideration of the factors we have emphasized, and because we believe it can be shown that his own view is incomplete without this, it may be allowed us to quote quite extensively and in abstract.

Lillie in his first paper finds that "the tendency of colloidal particles to collect at the electrodes indicates that they carry a surface charge, either positive or negative." Precipitation can be caused by ions bearing charges of opposite sign to those of the particles, liquefaction by those of like sign. "The researches of Picton and Linder, and Hardy indicate that the nucleo-proteids especially the chromatin of dividing cells and of spermatozoa are pronouncedly acid and therefore electronegative; cytoplasmic proteids are conversely basic and positive. Accordingly a difference of electrical potential exists between cytoplasm and chromatin in the cell, which difference is greatest at the time of mitosis, when the chromatin is most strongly acid. This potential difference may constitute the primary and determining condition of mitosis."

In confirmation of the correctness of this view he finds that "in all cases the appearance of the cytoplasmic radiations and the formation of the mitotic figure are accompanied by a passage of the nuclear chromatin into a phase rich in nucleic acid. Evidently the two parallel series of changes are intimately connected. Furthermore, the marked resemblance between the rays of the mitotic figure and the electric and magnetic lines of force are additional indications that the process is essentially electrical in nature. The position of the chromosomes during mitosis indicates a mutually repellent action similar to that of similarly charged bodies. The same action takes place in the chromatin filament whereby it assumes a coiled or spiral form."

In his second paper his purpose is to show the necessity of these electrical conditions for segmentation. Evidence for this is that "cytoplasmic cleavage in fertilized *Asterias* and *Arbacia* eggs is prevented in solutions of non-electrolytes, although the

[1] R. S. Lillie, *American Journal of Physiology*, VIII., IV., Jan. 1, 1903, and BIOLOGICAL BULLETIN, IV., 4, March, 1903.

nuclear division continues. In these solutions the electrolytes present in the egg must diffuse outward. Cleavage therefore depends on the presence of these in the cytoplasm. Likewise a strong tendency to fusion shows itself in blastomeres transferred to these solutions during early cleavage stages; therefore further cleavage also depends on the presence of electrolytes in the cytoplasm. This action is due to the ions into which they *dissociate*."

If all this is admitted it seems to us quite necessary to admit or infer therefrom that the presence of electrolytes in the eggs means also the presence of osmotic pressure; one cannot exist without the other; consequently their dissociation is identical with an increase in pressure. This would be in perfect agreement with our own theory, but is quite ignored by Lillie. He does, however, recognize with us the necessity of accepting a decrease and rearrangement of surface tension at cleavage, which he accounts for by a difference of electrical potential between the egg and medium. For "Lippman and Helmholtz have shown that the surface tension is greatest when the electrical potential at the boundary is zero, and decreases as the latter increases, for like electrical charges at the surface oppose the tension and diminish it. From this is evident the importance of electrolytes in segmentation; for the production of a potential difference between the egg and the medium separated by a semipermeable membrane is accompanied only by a migration of ions. Therefore, there are ions within the egg originally, and a potential difference implies that ions of like sign are respectively at the surface and in the interior. This state of affairs is found at cleavage. Observation of the direction of the fibrils in the egg during mitosis agrees with this view, *i. e.*, they correspond with the lines of force.' How is this difference of potential between the surface and the interior established? The answer is that "during segmentation especially is the chromatin markedly acid, the cytoplasm basic." This means in our opinion that there is chemical organization. "Agreeing with the results of Nernst and Olsen, the chromatin represents a charged body by the action of whose negative charges the anions are repelled toward the periphery, or the poles, and the kations are attracted to the nucleus." A curren

passes therefore in the direction of the gradient from periphery to center. Such inductive phenomena resulting from the increased acidity of the nucleus during mitosis must in turn be due, we find, to the preceding chemical changes resulting perhaps from the action of ferments. Accordingly, *it results* that "the center of the astral radiations is the region of highest positive potential, the surface of negative, and hence the decrease in the surface tension by the like charges present."

But a uniform decrease in tension is no change in form; to bring this about the tension must be unequal. To explain this, Lillie says, "There are indications, *e. g.*, the elongation of the spindle axis, that the primary lowering of surface tension by the above agencies is at the two sides of the egg opposite the astral centers. From the position of the astral centers during metaphase and telophase it is to be expected that the surface negative charges are densest near regions adjoining the long axis of the egg and that there surface tension is lowered to the greatest degree. The effect in these regions will increase as the daughter groups of chromosomes approach the poles, since the inductive action increases as the distance decreases. The surface tension at the regions adjoining the astral centers must therefore decrease as the daughter groups approach the surface, *i. e.*, the difference between the surface tension at the poles and at the equatorial region progressively increases. Eventually the egg is surrounded by an equatorial surface zone possessing higher tension and acting like a constricting band; a cleavage furrow follows."

"From the fact that not all of these events takes place when the egg is placed in solutions of non-electrolytes, it is clear that cleavage depends on the presence of ions, and that in fertilization the spermatozoön carries either these necessary electrolytes into the egg or ferments which initiate their formation and action."

That the acceptance of the events either identical with or similar to those so ingeniously outlined by Lillie is necessary in order to complete the theory of segmentation advanced in our own paper, may be admitted. But that the two are not contradictory to but rather must supplement each other seems quite as necessary to admit. The constricted form of segmentation must

be accounted for, but if this is done by electrolytic theories, then the existence of osmotic pressure cannot be denied. Lillie, however, neglects its consideration and in this respect his view is incomplete. If the necessity for segmentation of the presence of electrolytes in the egg is *proven* by such data as Lillie has advanced then osmotic phenomena must be also present, and that they play an important part cannot be denied in the face also of the evidence from artificial parthenogenetic methods. Rather, *conversely*, starting with the known fact of the effectiveness of, *e. g.*, the osmotic pressure methods, the possibility of these leading to the electrical changes should be shown. The objection may be made that, although present, osmotic pressure may nevertheless be left out of consideration for the reason that it is of low intensity. This objection does not hold good, we think; for, given a semipermeable membrane and the tendency of surface tension to reach its maximum, the latter will do this until balanced by at least as high a pressure as in the normal surrounding medium, sea water, of the marine forms we are considering.

It therefore seems possible and even necessary to unite the two views; to take the position that the electrical phenomena described by Lillie cannot be done away with but, being essential, they can nevertheless, as we have experimental evidence to show, be brought about in a number of different ways, such as amount of water present, action of heat, agitation, etc.; and that they coöperate with the factors we have emphasized in making up the physical and chemical events of cleavage processes. This possible perfect agreement of Lillie's theory with our own, into which all the factual methods have been shown to fit, adds, it seems to us, one more confirmatory element to the synthesis which it has been our purpose to attain.

The electrolytic methods which have been referred to as the *third* class which bring about physical changes may therefore be explained from the standpoint of this more complete view. As our factual result we have artificial parthenogenesis produced in *Asterias* eggs by the action of *N*/10 HCl solution (2–5 c.c. + 100 sea water) for about one hour, with a subsequent transferral to sea water. Dissociation takes place in the dilute solution used.

The negative results obtained with KCl indicate that it is the H and not the Cl ion that is effective. Two views of the way in which this ion acts are conceivable. First, an interpretation we have referred to previously, it may be that only the H ions penetrate the membrane, either because of *its* specific chemical nature or because of *their* greater diffusion velocity; or, second, that, with no penetration occurring, the cytoplasmic membrane simply attracts the H ions to the surface.

Both views however present difficulties if the H ions are considered to have a purely inductive (physical) action. The first view in its very genesis, because, *firstly*, unless the surface is negative all the time it is hard to understand why the positive ions should even be attracted to it, much less penetrate it; if, on the contrary, it is at first positive they would not reach it, being repelled, unless their velocity of diffusion overcame this repulsion; but *secondly*, because if penetration does occur it is difficult to understand how at least many of the ions can reach the nucleus from which point possibly to, in turn, induce negative charges at the surface; for cytoplasm and H ions are like-charged; these would, however, tend to cause a repulsion of cytoplasmic particles and therefore an increase in pressure. The second view meets with objections because, with *no penetration occurring*, it is difficult to understand also here why, firstly, if the surface is not yet negative before mitosis, either H or any other kations should be attracted, or, secondly, if the surface is negative at all times and the cytoplasm positive, so that the surrounding H ions may induce further negative surface charges, why the H and not other kations as well should be effective. That they are might seem to be explained by the comparative diffusion velocity of H, K and Cl ions (H is 313, Cl 65.9, K 63.8)[1] from which it might be assumed that the K ions are not effective because with them the Cl ions while in the case of HCl the H ions have the greater comparative velocity. But this explanation would not hold good for explaining positive results with $Ca(NO_3)$ on *Amphitritus*,[2] for the diffusion velocity of $Ca_{\frac{1}{2}}$ is 62, that of NO_3 65.[3]

[1] Höber, *loc. cit.*, p. 72.
[2] Fischer, *Am. Jour. of Physiology*, VII., III.
[3] Höber, *loc. cit.*, p. 191.

Between the difficulties of the two views it seems necessary to conclude that *certain* ions penetrate the membrane because of *their* and *its* specific chemical nature and of their greater comparative velocity and notwithstanding the possible repulsion, and that there follows a specific chemical effect on the chemically organized cell contents accompanied by those electrical and osmotic phenomena above considered. That there is a chemical effect is indicated also by the different results obtained in determining the rhythm of immunity of fertilized *Arbacia* eggs to ether, HCl, KCl, etc., an account of which will appear in a later paper. Furthermore, from this standpoint there should be in theory no effect resulting from the use of either the anodal or kathodal end of the current on the eggs of, *e. g.*, *Asterias*, and the negative results of such experiments carried on by the author this summer are confirmatory of this view.

Conclusions and Summary.

Under the experimentally justified assumption that the organism is a peculiar complex of energies, so that general physical principles are therefore valid for it, it is found that the effectiveness of both normal and artificial fertilization methods can be explained from one standpoint, viz., firstly, that the necessary condition for the event of cleavage, is the creation, previous to it, of an uncompensated potential difference between osmotic pressure and surface tension by increasing in a chemically organized egg either absolutely or relatively the pressure or by decreasing the surface tension; secondly, that the event of cleaving is itself identical with the equilibrating and compensating of this difference, which necessiates an average decrease in both the potentials, osmotic pressure, and surface tension; and thirdly, that there is an accompanying unequal distribution of electrical charges at the surface and at the center in such a way that constriction results therefrom.

This constitutes the synthesis which we purposed, and is offered only as an attempt, that, although in itself justifiable, presents much that is incomplete and tentative.

College of the City of New York,
Department of Philosophy, December 22, 1903.

EXPERIMENTS ON THE DEVELOPMENT OF EGG FRAGMENTS IN CEREBRATULUS.[1]

N. YATSU.

The question, "To what extent can the principle of germinal localization be applied to the unsegmented egg, and how far, on the other hand, may the specification of the embryonic regions be considered a progressive process that falls under the category of epigenetic phenomena?" (Wilson, 1903[2]) led me in the summer of 1903 to carry out a series of removal-experiments on the eggs of *Cerebratulus lacteus*, during the later part of my stay at the Tufts College Marine Laboratory at Harpswell, Me. I wished to determine whether the cytoplasmic localization is progressively established, and especially to ascertain the conditions existing in the egg just prior to the first cleavage. To this end I have examined the development of egg fragments, obtained by cutting, at four successive periods between discharge of the egg and the first cleavage, namely, (1) before the dissolution of the germinal vesicle, (2) at the metaphase of the first polar mitosis, (3) at the period of conjugation of the egg- and sperm-nuclei and (4) after the constriction of the first cleavage appeared. The result shows that the percentage of abnormal larvæ steadily increases as the egg approaches the two-cell stage. When I appreciated the importance of comparing with these results the development of isolated blastomeres of the two-cell stage, the breeding season was nearly over, and I was only able to make a few experiments, which are not numerous enough to give a satisfactory basis of comparison. I hope, however, to carry on more complete experiments at the first opportunity.

A word about cutting the eggs. A drop of water containing several eggs was spread on a slide, and some of the water was

[1] For the preparation of the present paper my best thanks are due to Professor E. B. Wilson for his kindly suggestions and criticisms. I am also indebted to Professor J. S. Kingsley for kindness shown me at the Harpswell Laboratory.

[2] E. B. Wilson, "Experiments on Cleavage and Localization in the Nemertine Egg," *Arch. f. Entwm.*, Bd. 16, Heft 3, p. 440.

removed by a pipette. The egg, thus flattened a little, was cut with a fine scalpel. It sometimes proved better, in order to spread the water evenly, to use a little albumen fixative, rubbed on the slide, and thoroughly washed off with a brush. In doing so, the quantity of glycerine left on the slide was infinitesimal, so that it did not affect at all the development of the eggs. My experiments consisted in cutting off a portion of cytoplasm from unsegmented eggs at the four periods already mentioned, in the first two cases fertilizing the fragments, and rearing the resulting embryos up to the pilidium. For the sake of uniformity I have confined my work to the nucleated fragments. Cleavage was studied up to the eight-cell stage. Among the pilidia thus obtained, I found many abnormal ones, and they were compared with the normal larvæ. Every pilidium was drawn with a camera between 48 and 58 hours after fertilization. During drawing they were kept still by sucking out some of the water from under the cover-glass, which was supported by a piece of thread. Thus they could barely move without distortion of their shape. It should here be noted that defective larvæ remain always defective ; moreover, the defective parts become more and more prominent as the larvæ grow. No size regulation takes place among the pilidia ; the smaller the original egg pieces, the smaller the pilidia.

Series A.

Development of Fragments obtained before the Disappearance of the Germinal Vesicle.

A portion of cytoplasm was cut off from the egg immediately after its release and while the germinal vesicle was intact, and the nucleated piece was fertilized. Care was taken not to injure the germinal vesicle, and most of the operations were done upon eggs in which it was eccentrically situated, so as to cut off as much cytoplasm as possible. Since the germinal vesicle, as a rule, lies nearer to the animal pole, it may be inferred that most of my sections were performed in the vegetative hemisphere, although I did not record the exact plane in this series.

Thirty-five egg-fragments were able to develop up to the pilidium stage, the rest having either died or been rejected on account of polyspermy. The result may be tabulated as follows :

Perfect pilidia 30	smaller 6	.....	85.7 per cent.
	larger 24		
Defective pilidia 5		.....	14.3 per cent.

It is striking that comparatively few larvæ turned out to be defective. All of these are shown in Fig. 1, *C–G*,[1] a glance at which shows that they are certainly abnormal, yet their defect is not so great as those obtained at later periods (Series B and C).

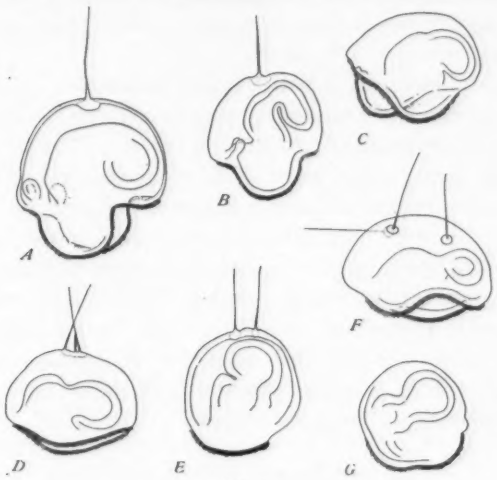


FIG. 1.

1*C* represents a larva which would be perfect, if there were an apical organ. 1*D* is another defective one with three apical organs, two tufts having been about to fuse; in other respects it is perfect. A pilidium represented in 1*E* is much more abnormal than the preceding two. It has two apical organs and a pair of ciliated lappets of smaller size. The shape of the gut also is not normal. Another pilidium (1*F*) resembles the one with three apical organs just described (1*D*), but differs from it in having three apical organs widely separated from one another — two on the left side, one on the right. In this larva the left ciliated lobe is indented. Lastly we have a very defective pilidium (1*G*), which, like 1*C*, has no apical organ. While the ciliated lobes

[1] All the figures throughout the paper have been drawn by the camera; 84 diameters magnification.

are comparatively normal, the gut is quite abnormal, the stomach being larger than the œsophagus, and the position of the mouth deviating from the normal. Nevertheless each part is complete, except the apical organ. It should not be overlooked that the apical organ is abnormal in every one of five defective pilidia, while both the gut and ciliated lobes are fairly unaffected by the operation. From this it is probable that the basis of the apical organ is vaguely foreshadowed as early as the stage at which the germinal vesicle is still intact.

It is highly important to note that the formation of a pilidium does not depend on the size of the piece, since one fourth of the perfect larvæ are decidedly smaller than the rest. The larva, 1*A*, is one of the larger group, and 1*B* one of the smaller. Both are perfect in every respect, but one is a little larger than one half of the other. From the fact that, in spite of cutting the eggs at random, a large percentage of perfect larvæ were produced, that the perfect ones vary greatly in size, and that the defects are not so considerable as at a later period, the most natural interpretation one can draw would be that, antecedent to the dissolution of the germinal vesicle, the egg cytoplasm still shows little or no definite specification of the germ regions.

Series B.

Development of Fragments obtained at the Metaphase of the first Polar Mitosis.

The eggs were cut when the mitotic figure of the first maturation division was completed; this can readily be seen as a clear space at the animal pole. The nucleated fragment was fertilized. Sixty-five eggs thus operated were able to develop into larvæ. Owing to a change of consistency of either the gelatinous egg envelope or the egg itself, I found it more difficult to operate at this stage than at any other. The result was:

Perfect pilidia 34	52.3 per cent.
Defective pilidia 31	47.7 per cent.

Notwithstanding the difficulty of the operation, it was comparatively easy to cut off a considerable part of cytoplasm from the egg of this stage on account of the peripheral position of the

mitotic figure. Consequently in some cases the fragments were quite small. In this series all the defective larvæ were more abnormal than in the preceding. The abnormalities may be classified as follows:

Defect in apical organ.	Larvæ with	supernumerary apical organ	4	14
	" "	no apical organ	10	
Defect in ciliated lobes.	" "	ciliated lobes indented or not well developed	16	27
	" "	only one ciliated lobe	11	
Defect in gut.	" "	no gut	6	21
	" "	no œsophagus	5	
	" "	no stomach	1	
	" "	stomach cut off from œsophagus	2	
	" "	gut not well developed	7	

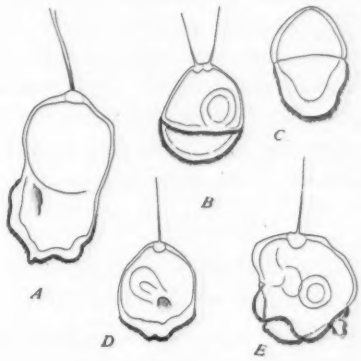


FIG. 2.

The disturbance of the apical organ (2*B* and 2*C*) is of special interest, indicating that its basis covers quite a large area over the animal pole, since in this series it was impossible to cut off cytoplasm from the top of the egg. While in Series A the defective part was chiefly restricted to the apical organ, we find that in the stage under consideration the abnormalities extend to the ciliated lobes and gut also. As will be seen from the above table, a large number of the defective pilidia have abnormal ciliated lobes; in some, one of the lobes is very short (2*B*), while in others the lobes are indented (2*E*), reminding us of *Pilidium branchiatum*. Quite commonly one lobe is entirely wanting (2*A*, 2*C* and 2*D*). The defect in the gut is remarkable. In a few larvæ

there is no gut at all, these being not far from the "Dauerblastulæ" (2*C*); in other cases the gut is represented by a shallow depression (2*A*). In some defective larvæ either the stomach or the œsophagus may be wanting (2*B*, 2*D*). In a few cases the stomach is cut off from the œsophagus (2*E*).

It is noteworthy that there occur a few defective larvæ which are as large as the perfect ones (2*A*). And among the perfect pilidia only three were smaller than the others.

From the increased number of defective larvæ in this series, it may be inferred that, since the fading of the germinal vesicle, the regional specification has advanced. But the occurrence of perfect pilidia equal in number to the defective ones cannot be overlooked. It is possible that the production of the perfect larvæ is because the injury was too small to have materially affected the organ-bases, or because the plane of section was such as not to disturb their normal proportions. The number obtained is, however, too great to lend much probability to either of these suggestions. A more likely interpretation is that the egg still possesses a considerable power of regulation.

Series C.

Development of Fragments obtained at the Period of Conjugation of the Egg- and Sperm-nuclei.

The eggs were fertilized and a portion of cytoplasm was cut off a little after the second polar body was extruded, *i. e.*, at about the stage at which the egg and sperm nuclei came to fuse. In this series the polar bodies gave a very good landmark for orientation of the egg. The accompanying cut shows the direction of section plane (Fig. 3). The eggs which were able to develop up to pilidium were not as numerous as in Series B, but they gave a fairly conclusive result, as shown in the table on p. 102.

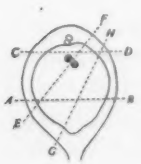


Fig. 3.

Most of the pilidia of this series are defective, as tabulated. Not only that, the defective parts correspond in a general way to the region from which the cytoplasm was cut.[1] It is very

[1] The apparent contradictory result as in the case of Nos. 17 and 23 is probably due to the volume of the cytoplasm cut off.

No. of Eggs.	Section Plane.	Condition.	Remarks.
1	AB	D(efective)	Ciliated lobes short.
2	"	D	No apical organ; ciliated lobes asymmetrical.
3	"	D	One of ciliated lobes wanting. Gut not well developed.
4	"	D	Apical organ on the right side.
5	"	P(erfect)	
6	CD	P	
7	"	P	
8	"	P	
9	"	P	
10[1]	"	D	Two apical organs. Gut double.
11	EF	D	Ciliated lobes defective.
12	"	D	One of ciliated lobes wanting.
13	"	D	One of ciliated lobes defective.
14	"	D	Gut defective.
14′	"	D	No apical organ; one ciliated lobe wanting; gut comparatively large.
15	"	D	One of ciliated lobes wanting.
15′	"	D	One of ciliated lobes wanting.
16	GH	P	
17	"	D	One of ciliated lobes wanting.
18	"	D	Neither of ciliated lobes developed, but the margin ciliated.
19	"	D	One of ciliated lobes wanting.
20	"	D	Ciliated lobes defective.
21	"	D	Ciliated lobes defective.
22	"	D	One of ciliated lobes wanting.
23[2]	"	D	No apical organ; only one lobe present; gut imperfect.
23′			No apical organ; only one lobe present; gut imperfect.

Summary:

Perfect pilidia 6.....24 %
Defective pilidia 20.....76 %

important to observe that most of the perfect larvæ were produced from the egg cut along *CD*. The basis of the apical organ must, therefore, have existed below the line *CD*. This fact, and the frequent occurrence of a defective apical organ in series *B* taken together, it may safely be concluded that the basis of this organ takes the form of a broad ring a little above the equator. But how it finally takes an apical position I am at a loss to imagine. Although Professor Wilson's experiments[3] were not directed to this problem, his results seem to tally well with

[1] In this egg the nuclei conjugated in the vegetative hemisphere; the section plane passed nearly through the equator.

[2] In this egg the sperm-nucleus must have been present in the piece which I thought enucleated.

[3] Wilson, *l. c.*, pp. 432, 433 (Fig. 10), 436.

my conclusion. He obtained through the section near the animal pole along *op* (his Fig. 3, *B*, on p. 240) the animal larva without any apical organ, the vegetative one in this case having been provided with the multiple apical organs (his Fig. 10, *E*, on p. 433). On the other hand, in another case, he found that both the animal and vegetative larvæ had the normal apical organ

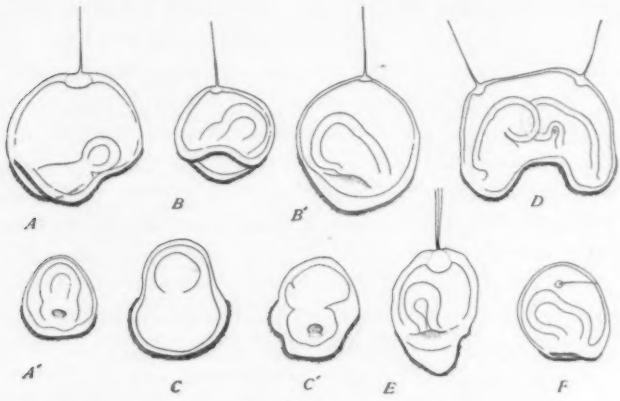


FIG. 4.

(his Fig. 10, *A* and *B*) when he cut along the plane near the equator (*kl* of his Fig. 3, *B*). In this case it may be inferred that the section plane bisected the basis of the apical organ. He gives still another instance of the animal larva with the apical organ (his Fig. 10, *C*) obtained when he cut below the equator along *mm* (his Fig. 3, *B*).

The pilidium No. 10 (4*D*) is a very important one. It was produced from an egg from which about one third of the animal hemisphere was cut off. This was about as large as a perfect larva if seen from the side, but it was extremely compressed laterally. It has not only two apical organs but two guts. I suspect that it might have been disturbed by some unknown cause at the two-cell stage because, as a rule, half-larvæ obtained by isolation of blastomeres are larger than one half of the normal larvæ. In another pilidium (No. 4) I found the apical organ shifted from the normal position (4*F*). In spite of this, it swam with the apical pole directed forward like a normal pilidium.

In three cases I got two larvæ from one fertilized egg. There are three possibilities to explain this: first, when the operation is done before the fusion of the egg- and sperm-nuclei; second, after the first cleavage mitosis came to the telophase; third, when the egg is doubly fertilized and the segmentation nucleus is cut apart from the sperm-nucleus. Since my operations were done not so late as the second case, the result may be due either to the first or the third cause. Whatever the cause may be, the comparison of the resulting larvæ is very interesting. $4A$ and $4A'$ (Nos. 14 and 14′) show a most instructive pair of pilidia. The larger of the two is almost normal, except that the gut is very defective, while the smaller one has a comparatively large gut. Professor Wilson found a similar pair of larvæ by cutting the blastulas (cf. his Fig. 11, *A* and *B*). Another pair ($4B$ and $4B'$, Nos. 15 and 15′) are also important; both of them are normal, but one ciliated lobe is wanting in each. In still another pair, $4C$ and $4C'$ (Nos. 23 and 23′), both are devoid of the apical organ and have a very defective gut. Either of them is barely more than the lappet.

Now let us see how the basis of the ciliated lobes and gut are disposed in the egg of this stage. Most of the defective larvæ have abnormalities in the ciliated lobes in some way or other. We can distinguish two kinds of defect; in one only one ciliated lobe has been developed, the other being entirely suppressed, so that the mouth can be seen from the side ($4A'$, $4B'$, $4C'$ and $4E$). The other kind of defect is shown in $4F$. In this case both the ciliated lobes are present, but they are very short and almost straight. This difference may be ascribed to the fact that the basis of the ciliated lobes is more or less bilaterally situated in the vegetative hemisphere near the equator. As for the basis of the gut I know very little, but it is certain that it lies near the vegative pole (cf. $4A$ and $4A'$).

Series D.

Development of Fragments obtained before the Completion of the first Cleavage.

The eggs were cut between the period of the appearance of the first cleavage furrow and the completion of the division. This

experiment was begun at the end of my stay at Harpswell, and I can give here only four cases.

(1) The egg was cut along *AB* (5*A*) and a perfect pilidium was produced which is represented in 5*C*. It is important to observe that, in spite of cutting off the cytoplasm from the animal pole, the apical organ has developed undisturbed.

(2 and 3) The eggs were cut along *CD* (5*A*). From each blastomere arose a pilidium with one ciliated lobe and no apical organ. The cases are too few to draw any conclusion, but the importance of the cytoplasmic bridge connecting the blastomeres and of constriction of the first cleavage upon the arrangement of organ bases, is not to be overlooked.

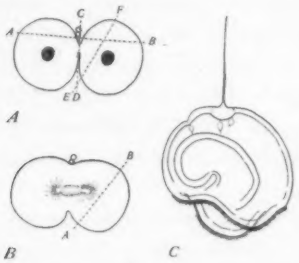


FIG. 5.

(4) The egg was cut along *EF* (5*A*). From the left half a perfect but dwarf pilidium resulted. This is a case worth describing, because the egg neither rounded up, as is usually the case, nor divided into two unequal halves, but soon after the cut surface was closed, the right half gradually increased in size accompanied by the decrease of the left half, and thus the egg was divided into two equal blastomeres.

SERIES E.

Development of the Blastomeres Isolated at the Two-cell Stage.

The blastomeres of eight eggs were separated at the two-cell stage when the cytoplasmic bridge had disappeared and the blastomeres had assumed a spherical form. From every blastomere a normal pilidium was developed. Some were about half the normal size, while some, for reasons that I cannot explain, were very

much smaller. It is remarkable that the development of the isolated blastomeres differs very widely from that of fragments of the period preceding. From a comparison with Series D it seems likely that the result may be different according to the period of cutting — *i. e.*, before or after the disappearance of the cytoplasmic bridge (cf. Series D, eggs *B* and *C*) and the pressing together of the blastomeres. In any event, further careful examination of the egg of this stage with reference to the question of egg specification is very desirable.

Conclusion.

From the above series of experiments we have seen that, if the cutting-off of a portion of cytoplasm is done before maturation, it only slightly affects the normal development; but if the operation is done at the first maturation stage the formation of the organs of the pilidia is considerably affected, and still more if it is done at the time of fusion of the germ-nuclei. We cannot, therefore, escape the conclusion that there must take place some progressive changes in the general make-up of the egg during the period extending from the time of dissolution of the germinal vesicle to the fusion of the germ nuclei. This period falls into two subdivisions, the first extending from the fading of the germinal vesicle to the metaphase of the first polar mitosis, and the second from the growing period of the sperm-nucleus to its conjugation with the egg-nucleus. The importance of these two periods has been correctly perceived by Delage (1901), Fischel (1903) and recently by Professor Wilson (1903). It is hardly necessary to state here that, although these two periods can be artificially separated by delaying fertilization, yet, under natural conditions, there is no pause between them, as the spermatozoön enters the egg before its maturation.

Let us next see what visible changes take place in the egg cytoplasm as the result of the above two acts, *i. e.*, the disappearance of the germinal vesicle and the entrance of the spermatozoön in the matured cytoplasm. As soon as the germinal vesicle breaks up, the nuclear fluids flow out and may diffuse through the egg. At this time currents may be formed carrying the egg cytoplasm from one spot to another. Although

in the *Cerebratulus* egg we have so far no direct evidence to support the occurrence of the last phenomenon, yet my observations on sections clearly show that a segregation of egg material does actually takes place at this period, the yolk accumulating in the lower hemisphere, while the clear and more finely granular protoplasm collects especially at the top of the egg, where, in the iron hematoxylin-Congo-red preparations, it stands out beautifully stained blue in contrast with the red yolk. After the fading of the germinal vesicle the eggs not only become fecundable, but also acquire much more power of forming cytasters than they had before. It is hardly necessary to state that in many forms remarkable changes take place in the egg at or after the entrance of the spermatozoön. It may further be pointed out, however, that the part played by the spermatozoön in causing rearrangements of the egg-substance is of a subsidiary character, as is shown in the case of parthenogenesis.

To the question as to what degree of localization exists in the cytoplasm of the *Cerebratulus* egg before the fading of the germinal vesicle, I am not in a position to answer, and it is almost impossible to find direct evidence. But the results of my experiments harmonize with Boveri's view that there is at first only a simple promorphological condition such as polarity and bilaterality, which may give a basis, so to speak, for a more definite grouping of material arising at the time of the flowing-out of the nuclear fluids. Otherwise it is impossible to understand the sudden increase in the proportion of abnormal embryos arising from the fragments obtained subsequent to the fading of the germinal vesicle. In the eggs of some forms (*e. g. Myzostoma*) the segregation of material has in a measure taken place long before maturation. In such cases the horizontal section of the egg before the disappearance of the germinal vesicle will, I think, produce a defective embryo. My observations on the later periods render it probable that at this time there must be a certain number of predetermined regions more or less firmly fixed, though this is not so clearly shown as in the ctenophore egg. It is extremely desirable to carry out careful studies with respect to the egg specification prior to the first cleavage in other forms.

So far as I could ascertain (up to the eight-cell stage), the cleav-

age of egg fragments was perfectly normal in Series A, B and C. It is especially noteworthy that the nucleated pieces of the fertilized eggs cleave exactly like a normal egg. Whatever amount of cytoplasm be cut off, the nucleated piece always divides at first into two and then into four equal blastomeres. Since these fragments so often gave rise to defective embryos, it is probable, not only that the two and four blastomeres differ from each other, but also that they do not correspond with those in the normal case. The same is true in the eight-cell stage, and so on. From this it seems further probable that cleavage of an egg takes its normal course irrespective of the localization of embryonic regions within. The cleavage pattern is stamped on the cytoplasm; but the end-result is governed by a different set of factors. The cleavage factors seem, therefore, to differ in this case from the morphogenic ones. "Though there is often a close and constant connection in the normal development between the process of cleavage and that of localization and differentiation, this connection is not a necessary relation" (Wilson, 1903[1]).

It is rather striking that, if the operation is done before the completion of the first cleavage, most of the egg fragments give rise to defective larvæ, while all of the isolated blastomeres at the two-cell stage develop into perfect pilidia (though the result is not conclusive, since my cases were too few). It is easy to conceive, however, that natural cleavage comes into operation in a quite different way from the artificial section, and it is probable that by the natural cleavage all the organ bases are equally distributed into two blastomeres which would be very unlikely after an artificial section.

Summary.

1. Before the germinal vesicle fades, there is no evidence of definite specification in the egg regions.

2. Dissolution of the germinal vesicle initiates the establishment of the germinal localization.

3. In the period between the entrance of the spermatozoön and the fusion of the germ nuclei, the localization becomes more definite.

4. The basis of the apical organ is not at the animal pole, but

[1] Wilson, *l. c.*, p. 439.

somewhere above the equator as a broad zone. The bases of the ciliated lobes and the gut lie mainly in the vegetative hemisphere.

5. The cleavage (up to eight-cell stage) is normal in an egg fragment obtained from the unsegmented egg, whatever be the amount of cytoplasm cut off, or at whatever period. It is probable that the cleavage factors do not here necessarily coincide with the morphogenic ones.

ZOÖLOGICAL LABORATORY, COLUMBIA UNIVERSITY,
October 29, 1903.

SOME OBSERVATIONS AND CONSIDERATIONS UPON THE MATURATION PHENOMENA OF THE GERM CELLS.[1]

THOS. H. MONTGOMERY, JR.

In a series of studies on the spermatogenesis of the Hemiptera, of *Peripatus*[3] and of the Amphibia,[4] I have endeavored to prove the following points:

1. That the chromosomes retain their individuality from generation to generation to that extent that a chromosome of one generation is not a new formation, however much its chemical substance has became changed by metabolism, but represents at least a part of a particular chromosome of the preceding generation.

2. That the first maturation mitosis in the species studied results in the separation from each other of entire univalent chromosomes, and hence is a transverse or reduction division; while the second maturation mitosis results in the longitudinal splitting of univalent chromosomes, and therefore is an equational division.

3. That the so-called "reduction in number" of the chromosomes is effected before the maturation divisions, by a pairing (conjugation) in the early growth period (synapsis stage) of the spermatocytes, of univalent chromosomes of like volume, each such composite chromosome then being bivalent with relation to the number in the spermatogonia.

4. That this conjugation of univalent chromosomes in the synapsis stage, is a conjugation of paternal with maternal chromo-

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 54.

[2] "The Spermatogenesis in Pentatoma up to the Formation of the Spermatid," *Zoolog. Jahrb.*, 12, 1898; "Chromatin Reduction in the Hemiptera: a Correction," *Zoolog. Anz.*, 22, 1899; "A Study of the Chromosomes of the Germ Cells of Metazoa," *Trans. Amer. Phil. Soc.*, 20, 1901; "Further Studies on the Chromosomes of the Hemiptera heteroptera," *Proc. Acad. Nat. Sci.*, Philadelphia, 1901.

[3] "The Spermatogenesis of Peripatus (Peripatopsis) balfouri up to the Formation of the Spermatid," *Zoolog. Jahrb.*, 14, 1900.

[4] "The Heterotypic Maturation Mitosis in Amphibia and its General Significance," BIOL. BULL., 4, 1903.

somes; and hence that the first maturation mitosis separates paternal from maternal chromosomes.

The third and fourth of these conclusions were new; the second has been a matter of much controversy, while the first has a considerable number of cytologists in its support. If these results can be generally established they will give a coherent basis for understanding the part played by the chromosomes in heredity, taken in conjunction with the important conclusion of Van Beneden[1] that the male pronucleus has a number of chromosomes equal to that in the female pronucleus; and of Henking[2] and O. Hertwig[3] that the maturation phenomena correspond very closely in the ovogenesis and spermatogenesis of the same species.

It is not my purpose here to go over the whole controversy and discuss in full the opposing views, for that has been done in my preceding papers, but rather to draw attention to a few of the more important results, and to add some new observations.

1. The First Maturation Mitosis in Amphibia.

Janssens and Dumez[4] have very recently reëxamined the spermatogenesis of Amphibia, particularly with regard to my results on *Plethodon* and *Desmognathus*, and decide that my position is both untenable and unproved: that the heterotypic mitosis is an equational division, and not a separation of entire univalent chromosomes as I had maintained. They state (p. 433): "On est tout étonné de ne trouver dans le texte de Thos. H. Montgomery *aucun argument* pour cette thèse." They do not mention my strongest argument at all, namely that the space enclosed by the heterotypic chromosome is a space separating two whole univalent chromosomes, and not a longitudinal split between two halves of a single chromosome, because this space is largest in the earliest stages, and not, as one would expect if it

[1] "Recherches sur la maturation de l'oeuf et la fécondation," *Arch. Biol.*, 5, 1883.

[2] "Ueber Spermatogenesis und deren Beziehungen zur Eientwicklung bei Pyrrhocoris apterus M.," *Zeit. wiss. Zool.*, 51, 1890.

[3] "Vergleich der Ei- und Samenbildung bei Nematoden," *Arch. mikr. Anat.* 36, 1890.

[4] "L'Elément nucléinien pendant les cinèses de maturation des spermatocytes chez Batrachoseps attenuatus et Pletodon cinereus," *La Cellule*, 20, 1903.

were a longitudinal split, smallest at those periods. In other words, I showed that at the earliest stage when the chromosomes can be distinguished, this space is largest, while the true longitudinal split is found in the axis of each arm of a heterotypic chromosome. They add: "L'auteur continue et, *sans en donner* la *moindre preuve* cette fois, que *les deux branches* de *l'anse s'enroulent l'une autour de l'autre* pour constituer les dyades enroulées, qui, d'après tous les auteurs, se trouvent dans les spermatocytes I avant la mise au fuseau des chromosomes." It is only necessary to rejoin that the comparison of the chromosomes as shown in my consecutive figures is sufficient proof. But when they say that my figures "sont fort schématisées," I simply answer that is not true, and that all were made with great care with the use of a camera lucida.

Janssens and Dumez do, however, bring up one good criticism. They note quite correctly that in the spermatogonia the chromosomes are of unequal lengths, while the two halves of a bivalent heterotypic chromosome are always of equal length. And they reason that if my view were correct that one of the heterotypic chromosomes is formed by the pairing of two univalent chromosomes, that I would have to demonstrate how two such conjugating chromosomes are always of the same length. For they argue that if the heterotypic chromosome were formed by a longitudinal splitting, such a splitting would fully explain the length equality of the two arms of the chromosome.

This is a good criticism, but I find it answered by a study of the relative volumes of the chromosomes in the equatorial plate stage of the spermatogonia. In every case where the pole view shows all the chromosomes lying in one plane, we can determine by carefully drawing them that there are just 12 pairs of chromosomes present, the two of each pair being of equal volume and frequently of similar form. One will find many cases where the pole view of a chromosome plate does not show this distinctly, but that is only when the chromosomes are irregularly arranged, and when their long axes do not lie in the plane of the equator.

A series of figures demonstrate this. Fig. 1 is the only case where all 24 chromosomes were seen in their entirety on pole

view; in Figs. 2–5 only such chromosomes were drawn as could be sent in their whole length; and in none of the figures were any chromosomes omitted that could be seen in their entirety. Two chromosomes of a corresponding volume are marked with the same letter, one in capitals and other in lower case, as, *e. g.*, *A* and *a*. All these cases were drawn, after numerous preliminary sketches, as accurately as possible with the camera lucida, and then those which seemed to correspond were lettered alike. Thus in Fig. 1, the large chromosomes *A* and *a* are alike, but differ in volume from all others; *B* and *b* are markedly alike, *C* and *c*, and all appear clearly paired. Sometimes, as in the case of *G*, *g*, *H*, *h*, the two of a pair appear dissimilar in the drawings, but this is simply because the curvature of one lies in a different plane from that of the other. Thus in Fig. 3 are shown also all 24 chromosomes, but the two marked *x* and *y* were seen so obliquely that it could not be determined whether they were alike. Fig. 6 shows a lateral view of a spermatogonic spindle, showing also a similar pairing of some of the chromosomes; and Fig. 7 shows the same phenomenon in an oblique pole view of a portion of one plate of daughter chromosomes of an early spermatogonic anaphase.

An examination of these Figs. 1–7, shows that the chromosomes are paired according to their volumes, that the two of a pair are very frequently of the same form; and that in most of the cases the two of a pair lie in the spindle close together. The only interpretation for this last condition is that corresponding chromosomes must have been arranged close together in the continuous chromatin spirem of the prophase, so that in the spirem *A* would be next to *a*, *B* to *b*, and so on. But on a study of nuclei in the late prophase (loose spirem) stage, I was not able to determine this positively, for until the chromosomes have shortened to their definitive forms, they are so irregular and twisted that it is practically impossible to determine their exact lengths on sections; probably crush preparations (such as those employed by Sutton) could be used with advantage here. At least there is no doubt of a continuous linin spirem in the spermatogonic prophase and of the arrangement of the chromosomes in a chain along this thread. And it is probable that

similar chromosomes are contiguous in this spirem, since in the metakinesis like chromosomes usually lie near each other; and, as Fig. 7 shows, they retain their contiguity even in the daughter cells.

Therefore we find, what Janssens and Dumez insisted that I should demonstrate, twelve pairs of chromosomes of the same length in the equatorial plate of the spermatogonia.

Now for the proof that such chromosomes unite into pairs in the spermatocytes, an explanation which, according to the Belgian cytologists, "est absolument fantastique et demanderait à être rigoureusement démontrée." On none of my preparations were there stages between the early anaphase of the last spermatogonic mitosis and the synapsis stage of the growth period. In the synapsis stage in *Desmognathus* the long and slender chromosomes are so intricately coiled together that it is impossible in sections to determine their exact relations. The cell has a distinct polarity, the nucleus at one end, at the opposite the greatest mass of cytoplasm containing the idiozome body. When the chromosomes begin to separate sufficiently for their boundaries to be distinguished, each appears as a loop or U with its ends at that part of the nucleus nearest the idiozome body. This is shown in Fig. 8, where only five of the loops are drawn in their entirety. On a transverse section of such a stage (Fig. 9) one finds 24 cross-cut portions of loop; every two of these portions correspond to the two arms of one of the U-shaped loop of Fig. 8. Therefore there are U-shaped chromosomes, with a particular arrangement, to the number of 12; hence they must be bivalent with regard to the 24 chromosomes of the spermatogonia. At this early stage (Fig. 8) there is no sign that the space enclosed by such a loop has arisen by a longitudinal splitting of a chromosome, for in fact the characteristic shape of the loops is the same now as at later stages (Figs. 10, 11). Now this was the main basis of my argument before — an argument that Janssens and Dumez ignore: that if this were a longitudinal split, we should find its commencement in such an early stage.

The true longitudinal split commences in the stage of Fig. 8, is very prominent in that of Figs. 9, 10; this is a clear splitting of each chromatin granule of the arms of each bivalent

chromosome, but the width of this split never becomes wider than that shown in Fig. 11. Janssens and Dumez, as all the workers on amphibian spermatogenesis before them, have entirely overlooked this split of each arm of a bivalent chromosome. With material stained in iron hæmatoxyline, and sufficiently destained, this split, though narrow, is perfectly distinct.

There is a point brought up by Janssens and Dumez to which attention must be drawn. They maintain (1) that there is no regular occurrence of a band of linin (that marked *k* in the figures of my previous paper), joining the two univalent arms composing a U, and placed at the bend of the U; and (2) that the linin spirem appears continuous and not broken into as many segments as there are U-shaped loops. A reëxamination shows me that they are right in regard to there being here a continuous linin spirem. And this is exactly what I gave especial study to proving to be the case in the corresponding stages of *Peripatus*. But I must maintain against these writers, that in *Plethodon* and *Desmognathus*, as in *Peripatus*, there is at no stage in the spermatocytes a continuous chromatin spirem. Sometimes one arm of a U-shaped chromosome seems to be continuous with the end of an arm of another, as in Fig. 11 (and Fig. 5 of my preceding paper). But this is unusual, and generally one finds, as in Figs. 8, 10 and 11, that the ends of the U-shaped chromatin loops are connected with the ends of other loops only by linin. Hence the boundaries of the bivalent chromosomes are perfectly distinguishable in most cases. As to the first point, I admit that the U-shaped loop, which I regard as composed of two univalent chromosomes attached at the bend of the U, does not show in all cases a band of linin at its bend; but it does nevertheless in many cases. However, on this point I did not place great insistence, as Janssens and Dumez maintain.

The U-shaped bivalent chromosomes of Fig. 11 shorten and condense into forms such as shown in Fig. 12. By the condensation of the chromatin the longitudinal split becomes hidden. This is not a remarkable phenomenon, as maintained by the Belgian writers; I and others have described it for frequent cases in arthropods. It reappears in the anaphase of the first maturation mitosis as a split along which the chromosomes

divide in the second mitosis. Finally the definitive shape is reached, as shown in Figs. 13 and 14. One peculiarity has often been described in heterotypic chromosomes: as they are placed in the spindle (Fig. 13), very frequently at the middle of each is one thickening, and this is more frequent than two thickenings. By comparison of a chromosome, such as that of Fig. 14, with earlier conditions, such as those of Fig. 12, it becomes evident that such a thickening corresponds to the separated ends of the U-shaped loop, which have finally came into close juxtaposition.

When we consider these points, we find two important facts: (1) that the twenty-four chromosomes are regularly paired in the spermatogonia, and that there the two of a pair lie close together; and (2) that there is no evidence that the space enclosed by any one of the twelve heterotypic chromosomes has been formed by a longitudinal splitting. In the spermatocytes there are twelve loops of the shape of a U or V. There is a longitudinal splitting, but along the long axis of each loop. The simple explanation of these facts is that in the spermatocytes, in the synapsis stage, there takes place the close conjugation of every two such chromosomes as were found in the spermatogonia; that two together constitute a U-shaped loop; and that therefore the first maturation division results in separating entire univalent chromosomes.

The difference of opinion between Janssens and Dumez and myself is more one of interpretation than of observation, though they did not notice the pairing of the chromosomes in the spermatogonia, nor yet the true longitudinal split. They frankly admit that by their interpretation the reduction in number of the chromosomes remains a mystery. They give no explanation of why there should be regularly disposed U-shaped loops. In assuming that a heterotypic chromosome has been formed by a longitudinal splitting, instead of by a junction of two univalent chromosomes, they contend for a kind of splitting very wide at the middle of the chromosome, but narrow at its ends; yet no such longitudinal splitting is known in any other case, and its difference is brought out sharply by comparison with the undoubted longitudinal splitting in the chromosomes of the later prophases of the spermatogonia. These heterotypic chromosomes

are different in form from others, just because they represent pairs of univalent chromosomes. If they arose by simple longitudinal splitting, why should they differ so in form from the chromosomes of the spermatogonia, or of the spermatocytes of the second order?

And here my thanks are due to Janssens and Dumez for this critique of my interpretation, because it induced me to study anew the amphibian spermatogenesis, and this reëxamination brought out the fact, strong in support of my position, that the chromosomes are regularly paired in the spermatogonia.

2. The Individuality of the Chromosomes.

By the idea of the maintenance of the chromosomal individuality we do not mean that a chromosome remains chemically unchanged from generation to generation (for after every mitosis a daughter chromosome grows to the size of a mother chromosome before it divides in the next mitosis), but that, despite great metabolic and structural changes, a chromosome of any generation is the descendant of a particular chromosome of the preceding generation, and is not a new formation. A chromosome of one generation represents a chromosome of a preceding, just as much as a cell of one generation represents a particular cell of a preceding generation. This idea was first propounded by Rabl,[1] and has steadily gained in support. The workers on ovogenesis have, for the most part, taken the position that it is not proved; but the reason there is simply the great duration of the growth period of the ovocytes, during a part of which chromosomal boundaries are not distinguishable. The students of spermatogenesis, on the other hand, are fairly unanimous in support of the view.

It is very important that this idea should be firmly established, and certain considerations would show it to be so. There is first the fact that from generation to generation the number of chromosomes remains the same, from the stage of the fertilized egg to that of the ovocyte or spermatocyte of the first order. Even the form of the chromosomes is maintained through these generations, as shown in the case of the cleavage of *Ascaris*. In the sperma-

[1] "Ueber Zelltheilung," *Morphol. Jahrb.*, 5, 1885.

togenesis of some Hemiptera there is no rest stage in the growth period of the spermatocytes, so that the chromosomes can be followed from the spermatogonia to the spermatids. In *Peripatus* this is but a short rest stage, and during it the boundaries of the chromosomes can be readily distinguished.

On the experimental side excellent evidence has been brought in support of this view, particularly by the study of abnormalities, by Boveri,[1] Zur Strassen,[2] Morgan,[3] and Herla.[4]

Evidence fully as strong as that from experimentaal study has been obtained by observations upon certain modified chromosomes of insects, which are:

3. The Heterochromosomes.

I offer this name to include those peculiarly modified chromosomes to which have been given the names "accessory chromosomes" by McClung,[5] "small chromosomes" by Paulmier[6] and "chromatin nuceoli" by myself. They have been described for the Hemiptera by Henking (*l. c.*), Paulmier, and myself; for the Orthoptera by Wilcox,[7] McClung, Sutton,[8] de Sinéty;[9] and for the spider by Miss Wallace.[10]

[1] "Zellen-Studien," *Zool. Jahrb.*, 1888; "Befruchtung," *Ergebn. Anat. Entw.*, 1891; "Ueber die Befruchtungs- und Entwickelungsfähigkeit kernloser Seeigel-Eier," *Arch. Entwickmech.*, 2, 1895; "Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns," *Verh. Phys. Ges. Würzburg*, 35, 1902.

[2] "Ueber die Riesenbildung bei Ascaris-Eiern," *Arch. Entwickmech.*, 7, 1898.

[3] "The Fertilization of Non-nucleated Fragments of Echinoderm Eggs," *Arch. Entwickmech.*, 2, 1895.

[4] "Étude des Variations de la Mitose Chez l'Ascaride Mégalocéphale," *Arch. Biol.*, 13, 1893.

[5] "A Peculiar Nuclear Element in the Male Reproductive Cells of Insects," *Zool. Bull.*, 1899; "The Spermatocyte Divisions of the Acrididæ," *Bull. Univ. Kansas*, 1900; "Notes on the Accessory Chromosome," *Anat. Anz.*, 20, 1901; "The Accessory Chromosome — Sex Determinant?" BIOL. BULL., 3, 1902; "The Spermatocyte Divisions of the Locustidæ," *Kansas Univ. Sci. Bull.*, 1, 1902.

[6] "The Spermatogenesis of Anasa tristis," *Journ. Morph.*, 15, 1899.

[7] "Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen," *Bull. Mus. Comp. Zool. Harvard*, 27, 1895.

[8] "The Spermatogonial Divisions of Brachystola magna," *Kansas Univ. Quarterly*, 9, 1900; "On the Morphology of the Chromosome Group in Brachystola magna," BIOL. BULL., 4, 1902.

[9] "Recherches sur la Biologie et l'Anatomie des Phasmes," *La Cellule*, 19, 1901.

[10] "The Accessory Chromosome in the Spider," *Anat. Anz.*, 18, 1900.

These are chromosomes which preserve to great extent their compact form during the whole growth period of the spermatocytes, and during the rest stages of the spermatogonia, and retain throughout this whole period the deep staining characteristic of the other chromosomes only during the height of mitosis. Thanks to this peculiarity they can be followed with extreme certainty from generation to generation, even during rest stages; and so are splendid evidence for the thesis of the individuality of the chromosomes.

Now there are two kinds of these. In the Orthoptera there is an unpaired one in the spermatogonia, larger than the other chromosomes; in the Hemiptera they are paired in the spermatogonia, and usually smaller than the other chromosomes. Otherwise in their behavior they are very similar in these two groups of insects. To include both these kinds the name "heterochromosomes," as expressing a difference from the other chromosomes, can be advantageously applied; and this would include (1) the "accessory chromosomes" (unpaired in the spermatogonia), and (2) "the chromatin nucleoli" or "small chromosomes" (paired in the spermatogonia). McClung regards them as sex determinants; I have considered them to be chromosomes that are in the process of disappearance, in the evolution of a higher to a lower chromosomal number.

Now these can be followed from generation to generation without in the Hemiptera undergoing those profound changes which characterize the other chromosomes after a mitosis. In the Figs. 15–23 they are the chromosomes marked *N*, *n*; Figs. 16 and 17 show them in the spermatogonic and first spermatocytic mitoses of *Anasa*; Figs. 18, 19 for the same stages in *Corizus;* Figs. 20, 21 for *Trichpepla;* and Figs. 22, 23 for the spermatogonic monaster and late prophase of the first maturation respectively of *Protenor*. In all these cases they can be recognized in mitosis by their much smaller size.

Recently I have found them to occur in the same number and form in the ovogonia; Fig. 15 shows a pole view of the chromosomal plate in the ovogonium of *Anasa;* Fig. 16 a similar view of the spermatogonium of the same species, and the chromosomes marked *N* and *n* are found to correspond exactly.

Now for *Protenor* I found that in the spermatogonic chromosomal plate there are always exactly thirteen elements (Fig. 22); the two smallest of these are the heterochromosomes marked *N*, *n*; they are paired, and in the following synapsis stage conjugate to form the smallest bivalent chromosome *N*, *n* of Fig. 23; these two chromosomes are then quite similar to the heterochromosomes (chromatin nucleoli) of the other Hemiptera. But there is a large element in the spermatogonium (*X*, Fig. 22), unpaired there, and which does not conjugate with any other chromosome during the synapsis stage, but remains unpaired in the spermatocyte (*X*, Fig. 23). This element I called the "chromosome *x*." Now, as McClung has also pointed out, this chromosome behaves exactly as does an accessory chromosome in the Orthoptera, being unpaired in the spermatogonia, and not conjugating with any other chromosome during the synapsis stage. Therefore in *Protenor* occur both kinds of heterochromosomes, the small paired ones, *N* and *n*, and the large unpaired one, *X*. *N* and *n* are "chromatin nucleoli" according to my terminology, while *X* is an "accessory chromosome"; thus both kinds of heterochromosomes occur in the same cell, and their likenesses and differences were fully discribed by me for this species. Both are recognizable through the whole growth period of the spermatocytes by their compact form and deep staining; but only the small pair, *N* and *n*, can be recognized in the rest stage of the spermatogonia. In three other Hemiptera, *Alydus*, *Harmostes* and *Œdancala* I showed that in the spermatogonia occurred also an uneven number (thirteen) of chromosomes; but in these the odd chromosome does not maintain its compact form during the growth period of the spermatocyte, and so is not recognizable there; but in the first maturation mitosis it is immediately recognizable as the only chromosome that has not conjugated with another to form a bivalent one. Now such a chromosome of *Alydus*, *Harmostes* and *Œdancala* agrees with the chromosome *X* of *Protenor* in not pairing with another during the synapsis stage; but differs from it in behaving like the other chromosomes after a mitosis, *i. e.*, in losing its compact structure and strong affinity for chromatin stains.

Why should a heterochromosome be sometimes unpaired in the

spermatogonia, sometimes paired? When they are unpaired they are larger than the other chromosomes. This might imply that such an unpaired heterochromosome really represents two in close union, *i. e.*, is already bivalent in the spermatogonium. And this I think is a very probable explanation, in view, first, of the behavior of the unpaired chromosomes during the growth period of the spermatocyte in *Protenor* (fully described in my paper, "The Germ Cells of the Metazoa"); and, second, of the fact that such a chromosome sometimes shows a distinct constriction at its middle (shown for *Harmostes* in my paper, "Further Studies on the Chromosomes," etc.).

It is hoped that these considerations, in endeavoring to show the likenesses and differences of the two kinds of heterochromosomes, will bring more uniformity in the interpretation of these modified chromosomes, and that such chromosomes should always be taken into account in any discussion of the idea of the individuality of the chromosomes.

4. The Conjugation of the Chromosomes in the Synapsis Stage, and its Relation to the Reduction Division.

It is now determined for a considerable number of cases that in the early portion of the growth period of both ovocytes and spermatocytes there takes place the process known as the "reduction in number" of the chromosomes. Thus if there are twenty-four chromosomes in the spermatogonium, twelve are found in the maturation period before the first mitosis. This fact was first established by Boveri ("Zellen-Studien," *l. c.*) and by Brauer.[1] Really the name applied is a misnomer, for there is no loss of chromosomes, no true "reduction" in this number, but it is a conjugation of the chromosomes. Rückert[2] sought to explain it by stating that in the prophase of the first maturation division the chromatin spirem breaks into only half the normal number of segments. This, however, is inadequate as an explanation, for I showed in the "Spermatogenesis of Peripatus" that in the prophases of the first maturation mitosis there is a

[1] "Zur Kenntniss der Spermatogenese von Ascaris megalocephala," *Arch. Mikr. Anat.*, 42, 1893.

[2] "Zur Eireifung bei Copepoden," *Anat. Hefte*, 4, 1894.

continuous *linin* spirem, which probably does not break into segments until the metakinesis of the first maturation mitosis, but no continuous *chromatin* spirem. Hence it is not a question of chromosomes which were already contiguous remaining contiguous, but of chromosomes which were first separated (except for their linin connections) conjugating to form pairs during the synapsis stage. The criterion of the synapsis stage is such a pairing; and the term "conjugation" of the chromosomes represents the facts much better than the term "reduction in number."

The bivalent chromosomes so formed by conjugation, in the Hemiptera, *Peripatus* and Amphibia, become so placed in the equator of the spindle of the first maturation mitosis, that entire univalent chromosomes become separated. This is a true reduction division in the sense of Weismann. Each spermatocyte of the second order thus receives whole univalent chromosomes in one half the normal (somatic) number. While the majority of writers still hold that no such reduction division occurs, the idea being abhorrent to them, there are still a number who have furnished an array of facts that can be interpreted only as speaking for such an occurrence; thus Rückert, Häcker, Vom Rath, Korschelt, Henking, Paulmier, McClung, Sutton, Nichols, Griffin, Van Winiwarter, Lillie, Schockaert. But the arraying of names on the one side against those on the other is no argument in itself, and we may pass to the discussion of certain facts which harmonize completely with the occurrence of a reduction division, and remain unexplainable on any other basis.

First, it may be recalled that there is a divergence of opinion as to which of the two maturation mitoses is the reduction division, some holding that it is the first and others, the second. There is no good reason, save the probability that there would be expected uniformity in such important processes, that this division should always be in the first mitosis, or always in the second; for it is quite possible that there is a difference in this regard in different objects. In the discussion which follows we will assume it to be the first maturation mitosis since there occurs the reduction division in the objects specially studied by me.

Now I reached the conclusion ("A Study of the Chromosomes," etc.) that in the synapsis stage there is effected a conjugation of paternal with maternal chromosomes; under "paternal" understanding those derived from the spermatozoön, and under "maternal," those from the ovotid. The arguments for this were stated as follows:

1. In *Ascaris megalocephala univalens* there is the normal number of two chromosomes. The ovotid and spermatid have each only one. In the fertilized egg there is one derived from the spermatid, one from the ovotid; therefore the bivalent chomosome found in the maturation period of the spermatocyte or ovocyte must have been formed by the conjugation of a paternal with a maternal chromosome.

2. In the spermatogenesis of the Hemiptera there are usually two small heterochromosomes in the spermatogonia. These unite to form a bivalent one in the spermatocyte. They become separated from each other in the first maturation mitosis so that no spermatid receives more than one. Evidently then in the fertilized ovum since only one comes from the spermatid, the other must come from the ovitid. Therefore in the conjugation of the two in the synapsis, it is a conjugation of a paternal one with a maternal one. That was reasoned out without any knowledge of such chromosomes of the ovogenesis. Now I add Fig. 15, showing among the chromosomes of an ovogenic monaster stage the two small elements *N* and *n*, which are heterochromosomes of the same number and size as those found in the spermatogonium (Fig. 16, *N*, *n*). Therefore, there must be a conjugation in the ovogenetic synapsis stage, as well as in the spermatogenetic, of a paternal heterochromosome with a maternal one.

3. That besides the heterochromosomes, whenever there is recognizable in the spermatogonic chromosomal plate a pair of chromosomes notably different from the others in volume, there is always found in the first maturation mitosis a particular bivalent chromosome notably different in volume from the other ones, and so evidently formed in the synapsis by the conjugation of the two peculiar univalent ones of the spermatogonium. This bivalent chromosome is so placed in the first maturation spindle

that its two univalent elements pass to opposite cells, so the sperspermatid has never more than one of them. One of those in the spermatogonium must accordingly have come from the spermatid and one from the ovotid, which combined to give rise to that spermatogonium. And here, also, in the formation of such a bivalent chromosome in the synapsis there must be a union of a paternal with a maternal chromosome. No other explanation seems possible.

These conclusions, the numerical ratios of certain clearly distinguishable chromosomes in the spermatogonia, to certain equally distinguishable ones in the spermatocytes and spermatids, could be established for the heterochromosomes for some forty species of Hemiptera, and for other chromosomes in the cases of *Trichpepla semivittata*, *Protenor belfragei*, *Peliopelta abbreviata*, *Prionidus cristatus*, *Zaitha fluminea* and *Corizus lateralis*. To make this point clear a few figures of certain of these cases are given here again. In the spermatogonium of *Anasa* (Fig. 16), as well as in the ovogonium (Fig. 15), are recognizable among the 22 chromosomes, two very much smaller than the others (*N*, *n*, heterochromosomes), and two considerably larger (*A*, *a*); in the first spermatocyte there are eleven bivalent ones, six of which are shown in Fig. 17, and here we recognize again the chromosomes *N*, *n* and *A*, *a*. In *Corizus*, in the spermatogonium (Fig. 18) are two particularly small (*N*, *n*) and two particularly large chromosomes; and these recognizable again in the first maturation spindle (Fig. 19). Similarly in the case of *Trichpepla* (Figs. 20, 21). In the spermatogonium of *Protenor* (Fig. 22) are thirteen chromosomes; a particularly large one (*X*), two next in size (*K*, *k*), and two smallest (*N*, *n*). In the spermatocyte (Fig. 23) *X* is recognizable as being the largest; it is the odd chromosome and is not paired with any other. *N* and *n* are paired and so are *K* and *k*.

From these observations I concluded that probably in every case the chromosomes in the synapsis united to form bivalent pairs in such a way that the one of each pair was paternal and the other maternal; and I was able actually to demonstrate it in those cases where the differences in volume between the chromosomes were sufficient to allow them to be followed from genera-

tion to generation. And I could also prove that in all cases the two components of each bivalent chromosome always become separated from each other in the first maturation mitosis.

Following this came the paper of Sutton (*l. c.*), proving conclusively that in the spermatogonium of *Brachystola* the chromosomes occur regularly in pairs of graduated sizes, the two of a pair being always of the same length; that in the synapsis stage bivalent chromosomes are produced by the conjugation of every two chromosomes of the same length; and that corresponding chromosomes became separated from each other in the reduction division (here the second maturation mitosis). So he concluded quite rightly that there are two series of chromosomes in the spermatogonium, a paternal series, *A*, *B*, *C* . . . *n*, and a maternal series, *a*, *b*, *c* . . . *n*, in which *A* corresponds to *a* in size and hereditary value, *B* to *b*, and so through the series. By *A* joining with *a* in the synapsis, like chromosomes conjugate; and by these separating from each other in the reduction divisions, it results that two chromosomes of like size are not found in the spermatid. Sutton was the first to demonstrate this for the whole series of chromosomes, and to argue that such a conjugation, together with the following reduction division, would operate so that no spermatid could receive two chromosomes of like hereditary value, but only one chromosome representing a particular value.

In the present paper I have shown that in *Plethodon* and *Desmognathus* also one may recognize the two corresponding series of chromosomes in the spermatogonium. An examination of *Ascaris megalocephala bivalens* shows the same relation. Pole views of the first cleavage spindle (Figs. 28–30) show each two larger (*A*, *a*) and two smaller chromosomes (*B*, *b*). The differences in size of the two pairs is not very great, but always recognizable when the chromosomes can be seen in their entirety. This is then evidently a case parallel to those described above: that of the larger pair (*A*, *a*) one is paternal and the other maternal, and that of the smaller pair (*B*, *b*) the same relation holds. Now the formation of the tetrads in the ovogenesis of this species has been described by Boveri as two equation (longitudinal) divisions of each bivalent chromosome; and Braur has reached the same result for the formation of the tetrads in the spermatogen-

esis. But in concluding this these writers do not give a satisfactory explanation either why or how univalent chromosomes unite to form bivalent ones, and so give no clue to a reason for the chromosomes being paired in the fertilized egg. This point can be settled only by a careful reëxamination of the changes in the early growth period, and here I shall simply call attention to certain appearances that speak for the first maturation mitosis in *Ascaris* being a reduction division.

In the fertilized egg are two pairs of chromosomes, *A*, *a* and *B*, *b* (Figs. 28–30), one pair being considerably larger than the other, and the two composing a pair sometimes differing somewhat in length but being approximately equal in volume. As Van Beneden first showed, two of these chromosomes come from the ovotid, and two from the spermatozoön. Fig. 27 shows a slightly earlier stage, the chromosomes in two groups, one group derived from the male pronucleus and the other from the female pronucleus. In the group to the right is a large and a small chromosome (*A*, *B*); in the group to the left also a large and small one (*a*, *b*). But *A* corresponds approximately to *a* in volume, and *B* to *b*. Therefore we may say that of the four chromosomes in the fertilized egg (Figs. 27–30) a small paternal one (from the male pronucleus) corresponds in volume to a small maternal one (from the female pronucleus), and a large paternal one to a large maternal one. In other words, of each pair of chromosomes in the fertilized egg, one chromosome is paternal and one maternal. Which two come from the male pronucleus, and which two from the female pronucleus, there is as yet no means of deciding, for the two pronuclei appear structurally alike. But to make my argument clear I will assume that *A* and *B* are paternal, and *a* and *b* maternal.

Now in the formation of the first polar body (Figs. 24, 25) we find the two well-known quadripartite chromosomes. There are two, not four; hence they are bivalent with regard to the normal number. In each bivalent chromosome (tetrad) we should expect then to find two univalent chromosomes. Both these figures (24, 25) were drawn with great care to get the exact proportions of the parts of the chromosomes; both represent the stage where one plate of chromosomes is passing into the polar body.

Now in each of these cases we notice in the polar body, as in the egg, a larger and a smaller bipartite chromosome. Thus in the polar body the larger dyad *A*, *A*, and the smaller *B*, *B*; in the egg the larger dyad *a*, *a*, and the smaller, *b*, *b*. As far as I can determine this relation appears to be constant: one large and one small dyad in the polar body as well as in the egg; and not two smaller (or larger) dyads in the polar body and two larger (or smaller) dyads in the egg. Now from what we have found to be the case in other objects, I would judge *A*, *A* to be an entire univalent chromosome that had been paired previously with the entire univalent chromosome *a*, *a*; and that a similar relation holds between *B*, *B* and *b*, *b*. This would then be a reduction division, separating entire univalent chromosomes. In favor of this is the fact that *A*, *A* is approximately similar in volume to *a*, *a*, and *B*, *B* to *b*, *b*; and we have learned that chromosomes of similar volumes conjugate in synapsis. Two dyads are left in the egg, *a*, *a* and *b*, *b*, each of which could be regarded as a longitudinally split univalent chromosome. In the formation of the second pole body (Fig. 26) the two parts of each dyad separate from each other, and this would be an equational division. There are then left in the egg the two chromosomes *a* and *b*, which differ markedly in volume. And this is in exact accord with the fact shown in Fig. 27, that from each pronucleus comes one large and one small chromosome.

This interpretation would bring *Ascaris* into close agreement with the other objects discussed in this paper: it explains why there are two large and two small chromosomes in the fertilized egg; why each pronucleus has one large and one small chromosome; finally, why the two bivalent chromosomes of the first maturation mitosis differ in volume. The idea that each such bivalent chromosome has been formed by a double longitudinal splitting, hence that both divisions are equational, gives no explanation for any of these phenomena, nor yet explains how or why bivalent chromosomes should be formed. The onus no longer rests upon us to prove the occurrence of a reduction division; but upon those of the other school to prove that a bivalent chromosome represents one chromosome that has undergone a double longitudinal division, and to show that such an interpre-

tation is explanatory of the kind of phenomena that we have discussed.

5. Chromosomal Combinations and the Mendelian Ratio.

In his paper on "The Chromosomes in Heredity"[1] Sutton argues that the combination of paternal and maternal chromosomes in the fertilized egg would result in a Mendelian ratio. It will be recalled that Mendel[2] in his experiments on crossing varieties of *Pisum*, to determine the law of transmission o parental characters to the hybrid, found the crosses to result in the ratio 1 *D*: 2 *Dr*: 1 *r*, in which *D* represents the pure character of one parent, *r* the pure character of the other parent, and *Dr* represents the possession of both characters. In other words: out of four offspring resulting from such a cross, one would resemble the father, one the mother, and two combine the characters of both parents.

Sutton starts with the fact that there are two series of chromosomes in each fertilized egg, *A*, *B*, *C*, ... *n* and *a*, *b*, *c*, ... *n*, the first set of paternal origin (from the spermatozoön) and the second of maternal (from the ovotid). In these series *A* is the homologue of *a*, *B* of *b*, and so on. In the synapsis stage of the germ cells *A* would conjugate with *a*, *B* with *b*, and so on, so there would be formed the bivalent chromosomes *Aa*, *Bb*, *Cc*, ... *n*. In the reduction division *A* and *a* would pass to separate cells, and such would be the case with *B* and *b* and the remaining paired chromosomes.

Then Sutton takes the case where there are the two homologous chromosomes *A* and *a* in an ovogonium and *A* and *a* in a spermatogonium; in the maturation period there would be formed *Aa* in the ovocyte, and *Aa* in the spermatocyte; the ovotids would contain then either *A* or *a*, and the spermatids either *A* or *a*. There would then result in fertilization these combinations:

$$A\,♂ + A\,♀ = AA$$
$$A\,♂ + a\,♀ = Aa$$
$$a\,♂ + A\,♀ = aA$$
$$a\,♂ + a\,♀ = aa.$$

[1] Biolog. Bull., 4, 1903.

[2] "Versuche über Pflanzenhybriden," *Verh. nat. Ver. Brünn*, 4, 1865.

"Since the second and third of these are alike the result would be expressed be the formula $AA : 2Aa : aa$ which is the same as that given for any character in a Mendelian case."

But as a matter of fact this can be so only in a case where there are only two chromosomes in the fertilized egg. For let us take the case where the normal number of chromosomes is four; express by capital letters those chromosomes originally derived from the spermatozoön, and by small letters those derived from the ovotid; and assume that A is homologous to a, and B to b. Then the spermatogonium would have the chromosomes, A, a, B, b, and the ovogonium have also A, a, B, b. By the synapsis stage would be formed bivalent chromosomes Aa, Bb in the spermatocyte, and Aa, Bb in the ovocyte. The reduction division would separate A from a and B from b in both spermatogenesis and ovogenesis. The spermatids would contain then either A, B, or a, b, or A, b, or a, B; and the ovotids either A, B or a, b, or A, b, or a, B. In the fertilization of one of these ovotids by one of the spermatozoa, 16 different combinations are possible: A, B, A, B; A, B, a, b; A, B, A, b; A, B, a, B; a, b, A, B; a, b, a, b; a, b, A, b; a, b, a, B; A, b, A, B; A, b, a, b; A, b, A, b; A, b, a, B; a, B, A, B; a, B, a, b; a, B, A, b; a, B, a, B. But only one of these combinations is of purely paternal chromosomes, namely A, B, A, B; and only one of purely maternal, namely a, b, a, b. The other fourteen combinations show paternal together with maternal chromosomes (six cases where the paternal and maternal chromosomes are present in equal number, four cases where there are three paternal chromosomes to one maternal, and four cases where there are three maternal chromosomes to one paternal).

Hence the ratio is : $1P : 14PM : 1M$, where P stands for purely paternal chromosomes, M for purely maternal, and PM for combinations of paternal and maternal chromosomes. This is clearly not a Mendelian ratio of 1 : 2 : 1. And obviously the disparity would become greater with any increase in the number of chromosomes. According to Sutton's own computation, in forms which have 24 chromosomes, the number of possible combinations of these in the fertilized egg would be 16,777,216. That would give the ratio of $1P : 16{,}777{,}214PM : 1M$.

But though the combinations of paternal and maternal chromosomes in the fertilized egg do not support the Mendelian ratio for hybrids, I fully agree with Sutton that "the phenomena of germ-cell divisions and of heredity are seen to have the essential features, viz., purity of units (chromosomes, characters) and the independent transmission of the same."

UNIVERSITY OF TEXAS,
November 29, 1903.

Explanation of Figures.

Figs. 1–3. Pole views of spermatogonic monasters of *Plethodon cinereus*.

Fig. 4. Pole view of a spermatogonic monaster of *Diemyctilus virescens*.

Figs. 5, 6. Pole and lateral views respectively of spermatogonic monasters of *Desmognathus fuscus*.

Fig. 7. Oblique pole view of one plate of daughter chromosomes, early spermatogonic anaphase of *Desmognathus fuscus*.

Fig. 8. Lateral view of a late synapsis (postsynapsis) stage of *Desmognathus fuscus;* four entire bivalent chromosomes shown, and half of another. Nuclear membrane not yet formed.

Fig. 9. Polar view of a spermatocytic nucleus at a slightly later stage in the same species.

Figs. 10–12. Successive prophases of the first maturation mitosis in *Desmognathus fuscus*.

Fig. 13. Lateral view of a spindle of the first maturation mitosis, showing three bivalent chromosomes in metakinesis; *Plethodon cinereus*.

Fig. 14. A heterotypic chromosome of *Plethodon cinereus* in its definitive form.

Fig. 15. *Anasa* (undetermined species from California), pole view of ovogonic monaster.

Fig. 16. *Anasa* sp., pole view of spermatogonic monaster.

Fig. 17. *Anasa* sp., lateral view of first maturation mitosis, showing six of the eleven bivalent chromosomes.

Fig. 18. *Corizus alternatus*, pole view of the spermatogonic monaster.

Fig. 19. *Corizus alternatus*, lateral view of first maturation mitosis, showing five of the bivalent chromosomes.

Fig. 20. *Trichpepla semivittata*, pole view of spermatogonic monaster.

Fig. 21. *Trichpepla semivittata*, lateral view of first maturation mitosis, showing all the bivalent chromosomes.

Fig. 22. *Protenor belfragei*, pole view of spermatogonic monaster.

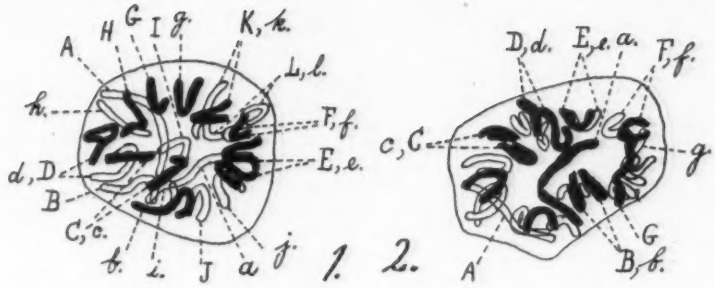
Fig. 23. *Protenor belfragei*, late prophase of the first maturation mitosis.

Figs. 24, 25. *Ascaris megalocephala bivalens*, formation of the first polar body (first maturation mitosis), the spindle seen very obliquely in Fig. 25.

Fig. 26. *Idem*, formation of second polar body.

Fig. 27. *Idem*, lateral view of the first cleavage spindle showing the two groups of chromosomes.

Fig. 28–30. *Idem*, pole views of the chromosomes in the first cleavage spindle.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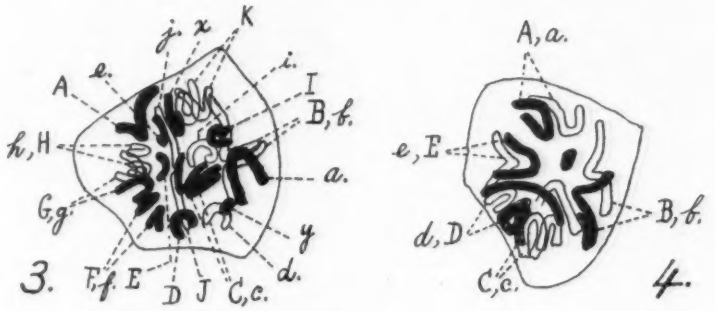
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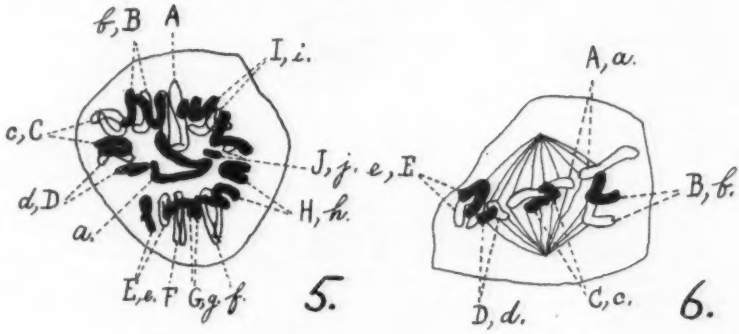
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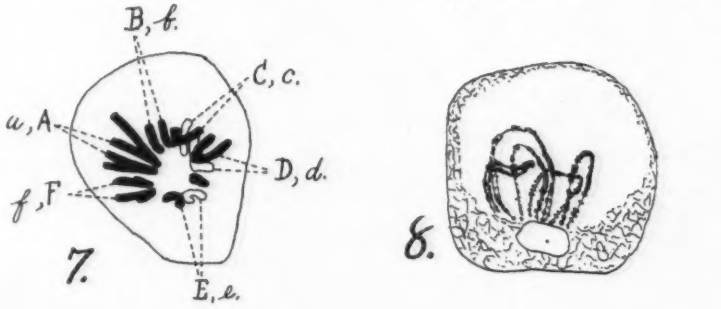
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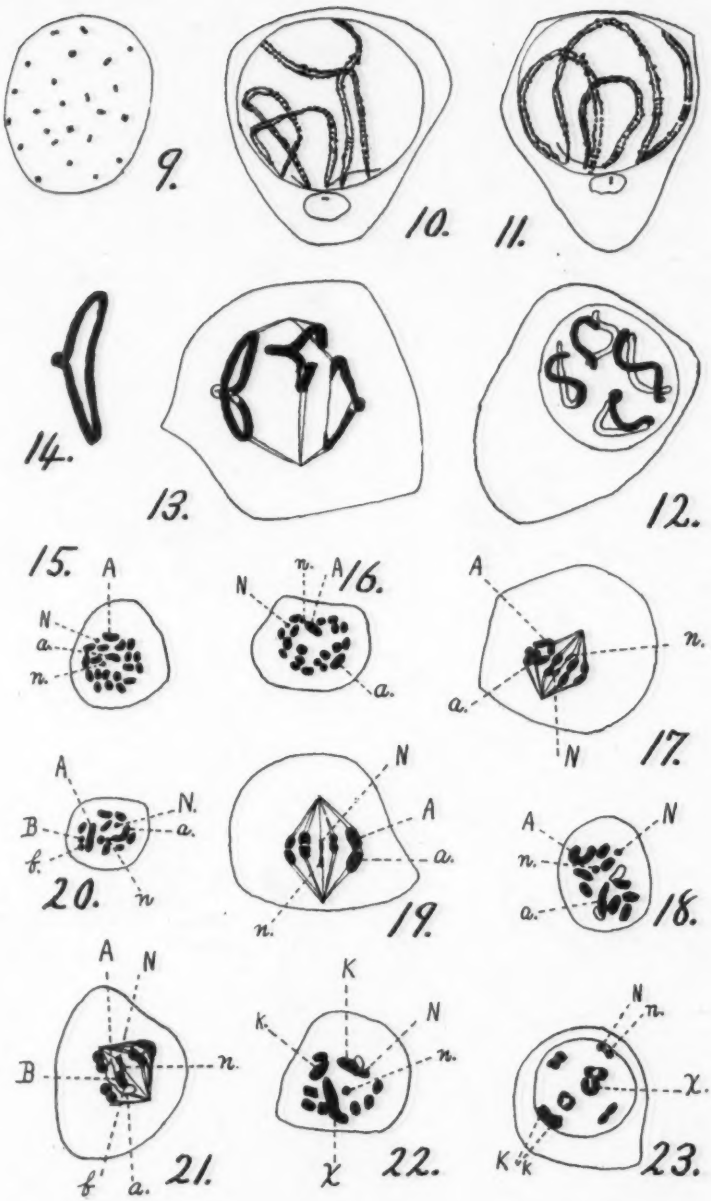
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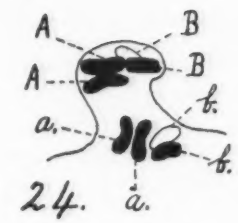

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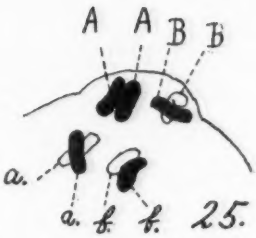

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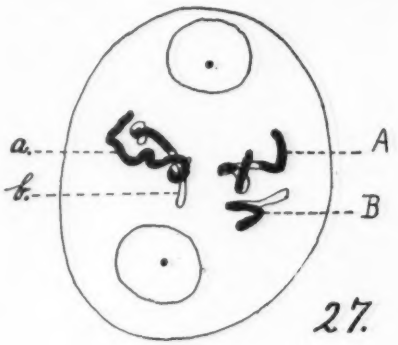

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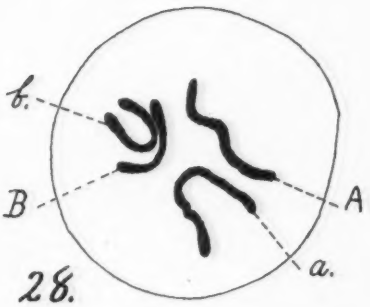

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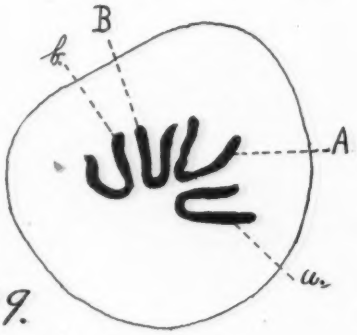

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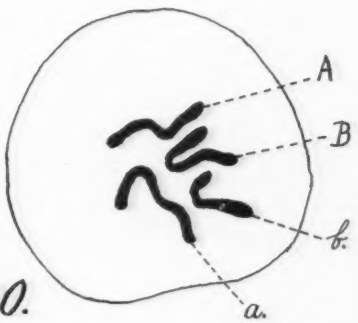

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